Auto Clave Day – Training

June 2nd, 2016
Project # 1
From Page # 1
To Page # 14
Originally Recorded by: Danny Dooling

Purpose: Preparation and plating of Agar and LB Broth

Materials:
62.5g LB Broth & 400 uL Chloramphenicol(CM34 34 mg/ml) & 7.5g Agar & Wooden applicator & a sample of Genehog & 2,500ml of Nano Water & 500ml glass Bottle & 25 Petri Dishes

Procedure:
1) Create LB Broth solution
   -Add 12.5g LB Broth powder to a 500ml glass bottle
   -Add 500ml of purified Nano Water to LB Broth Powder
2) Create LB Broth/Agar solution
   -Add 12.5g LB Broth powder to a 500ml glass bottle
   -Add 7.5g of Agar Powder to LB Broth Powder
   -Add 500ml of purified Nano Water to LB Broth Powder and Agar
3) Autoclave both solutions – (One tray - Cycle 1; Two or more trays – Cycle 2)
4) Autoclave Tips, Tubes and cylinders
5) Streak: Used bacteria *Genehog*
6) Place in 37degC overnight
7) Results tomorrow

To Create NEW Plates:
8) Obtain New Agar
9) Place Agar in microwave for 15 minutes at Power Level 3
10) Pour needed amount of Agar into a separate container
11) Add any antibiotics to your separate container (See Common Antibiotic Ratio below in Data)
12) Pour Agar into petri dishes so that it completely covers the bottom (Approx. ~20ml)
    - Don't get any Agar on the lid as that can lead to contamination
13) Place in 4degC until further use
14) LABEL LABEL LABEL!!!!!!!

Data: Below are the Common Antibiotic Ratio: (Step 1)

Antibiotics	Dilution
Chloramphenicol	x1000
Kanamycin	x1000
Tetracycline	x400
Ampicillin	x1000

Results: June 3rd, 2016

Of the two Colonies created, one, without CM34, yielded single colonies that we will use in the next round.

E-Coli Chemical Bacteria Cells in Liquid Media / Competent Cells Preparation – Training

June 3rd, 2016
Project # 1
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To Page #14
Originally Recorded by: Brynne Schwabauer

Purpose: Inoculate bacteria cells in liquid media

Notes: This is the training crash course to create C.C. cells. When you are actually creating new C.C. cells there are a few changes that need to be made.

- **At Step 3:** Place culture tubes in shaker overnight instead of for 2 hours
- **At Step 5:** When adding LB Broth do it in a 1:100 dilution instead 25 ml

Materials:
4ml Broth & 50ml Conical tubes & 2 Large Glass Culture tubes & 4 Cuvette &
2.5ml of Calcium Chloride Magnesium Buffer (CCMB) & Wooden Applicator &
125 ml Erlenmeyer flask & 6uL Test tubes

Procedure:

1) Add 2ml LB Broth solution media to large glass culture tubes
2) Use wooden applicator to transfer colonies obtained in Autoclave Training Day to the liquid media in tube.
3) Add large glass culture tubes to shaker for 2hrs (Begin at 9:17am: @37degC @250rpm)
4) Remove test tubes from shaker
5) Add 25ml of LB Broth to a clean 125ml erlenmeyer flask
6) To the same erlenmeyer flask add the LB Broth/ Colonies mixture (1 Tube)
7) Add erlenmeyer flask to Shaker for 2.5hrs (Begin at 11:30 am: **@30degC** @250rpm)
8) Add 1 ml of the LB Broth/ Colonies mixture into a cuvette
9) Add 1ml of water into a cuvette
10) Read the levels on the Cell Density Meter (Between 0.40 -0.60 Abs)
11) Repeat steps 4-6 (If not between desired values)
12) Transfer contents of erlenmeyer flask to 50ml conical vial
13) Place in Centrifuge for 10min (@4degC @ 5,000rpm)
14) Remove from Centrifuge and separate LB Broth from bacteria
15) Add 1 ml of CCMB (Resuspend with vortex)
16) Centrifuge again for 10min (@4degC @ 5,000rpm)
17) Remove and suspend bacteria in 2.5g/ml of CCMB (1:25 amount CCMB to total volume i.e. 4ml CCMB for 100ml of volume)
18) Aliquot into eppendorf tubes (40uL each)
19) Place in -80degC fridge over the weekend

Results: June 6th, 2016
Cells were ready for the next step of adding the plasmid. (See June 6th, 2016)

Transformation of Chemical Competent Cells with Plasmid DNA– Training

June 6th, 2016
Project # 1
From Page # 1
To Page #14
Originally Recorded by: Madison Bierman

Purpose: Introduce plasmid containing CM34 resistance genes to C.C. E Coli

Notes: Streaking for Plasmid, Glass beads for Ligation product

Materials:
Materials used for plating & 2uL Broth & 80uL Genehog cells & 0.6ml Eppendorf tubes (4x)

Procedure:
1) Transfer 20uL of the cells (from June 3rd, 2016 from -80degC freezer) into eppendorf tubes
2) Add 1uL of the Plasmid to each tube
3) Stir gently (vigorous stirring may change C.C. cells)
4) Chill tubes for 10 minutes on ice
5) Heat tubes for 35sec at 42degC (Heat Shock)
6) Chill tubes for 2 minutes on ice (Immediately)
7) Add 300uL LB Broth in each
8) Add tubes to Shaker for 1hr (@37degC @250rpm)
9) Warm up plates in 37degC incubator
10) Remove and plate either by streaking or glass beads
11) Place in refrigerator over night (4degC)

When Streaking:
- If we have a lower concentration of cells aliquot 50uL of DNA / Plasmid onto the plate, then proceed to Streaking. Do not dip Wooden Applicator.
- If we have a higher concentration of cells, like a glycerol stock or overnight inoculation, then simply dipping the wooden applicator and streaking is acceptable.

Results: June 7th, 2016

Cells were successfully transformed into competent cells (See June 8th , 2016)
The Streaking method gave more single colonies than the beading method.

Polymerase Chain Reaction (PCR) – Training

June 7th, 2016
Project # 1
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To Page #14
Originally Recorded by: Therese Ninh

Purpose: To clone PET30b –mCherry DNA

Materials:
5 uL of buffer & 4 PCR tubes & 3uL (25mm) of MgSO4 & 1uL of KOD POI & 5uL of dNTPs
10 ug/uL of PET30b –mCherry (2x) & 64.5uL of Autoclave water &
0.75uL of SLIC – NdeI-mCherry-F(20uL) & 0.75uL of SLIC – SacI-mCherry-R(20uL)

Procedure:
1) To Create Diluted SLIC primer: Add together
   - 2uL of NdeI-mCherry-F(20uL) from 100 uM Stock
   - 2uL of SacI-mCherry-R(20uL) from 100 uM Stock (10uM final mixture)
   - 16 uL of autoclave water
2) For the PCR add together in a PCR tube: Final Volume 25 uL
   - 0.75 uL diluted SLIC primer: Kept at -20degC
   - 2.5 uL of buffer (Kept at -20degC)
   - 1.5uL (25mm) of MgSO4 (Kept at -20degC)
   - 0.5uL of KOD POI (Kept at -20degC)
   - 2.5uL of dNTPs (Kept at -20degC)
   - 1 uL of PET30b –mCherry
     - (If using Chromosomal DNA: use 50ng instead)
   - 16.25uL of Autoclave water
3) Gently mix the Eppendorf tube
4) Place in PCR machine; (See settings for PCR in Data below)

Data: Settings for PCR (Step 4):

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	57	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	12 sec	29 times	3 min	inf.

Results: See DNA Purification: June 7th, 2016

DNA purification by Electrophoresis – Training

June 7th, 2016
Project # 1
From Page # 1
To Page #14
Originally Recorded by: Therese Ninh

Purpose: To prepare Agarose gel for Electrophoresis

Materials:
5 uL of buffer & Erlenmeyer flask & 2uL of Ethidium Bromide & 0.75g of low E.E.O. Agarose & 50ml of Electrophoresis Buffer & 5uL Ladder DNA & Calculated dye amount & 5uL test DNA

Procedure:

1) To prepare the Agarose gel:
   -Add together in an erlenmeyer flask and heat (in microwave)
   - 50ml of electrophoresis buffer
   - 0.75g of low E.E.O. Agarose (1.5% of buffer volume)
2) Place Agarose gel in the microwave for 2min at Power level 10
3) Once cooled to just above room temp. add 2uL of Ethidium Bromide to the flask
4) Pour solution in to an electrophoresis plate; add combs and wait to solidify (30-40 min)
5) Once the mixture has solidified remove the combs
6) Immerse plate in fresh buffer in the electrophoresis apparatus
7) Mix test DNA with dye (Dye is 1/6th total volume: Divide total volume by 5, that is how much dye needs to be added)
8) Pipe 5uL of Ladder DNA and test DNA into separate wells (Wells can hold up to 15uL each)
9) Plug in apparatus and leave on for 35min (100 volts)
10) Remove gel from apparatus
11) IF cutting out DNA pieces, then see Molecular Cloning /DNA Purification by Electrophoresis / SLIC Cloning / Transformation (6/9/2016: Step 5)
12) Take pictures using the BioRad Molecular imager (See settings for BioRad in Data below)
13) Compare results of Ladder DNA to our DNA samples
14) IF NOT cutting out DNA pieces, then throw away used gel after pictures are taken

Procedure for the creation of 1KB of DNA Ladder:

1) To prepare Ladder, add together
   -100 uL of Premade DNA Ladder
   -100 uL of Loading Dye
   -500 uL of Deionized Water

Data: Settings for BioRad Molecular imager: (Step 11)

- Gel Imaging
- Nucleic Acid
- Ethidium Bromide
- Check Filter

Results: June 7th, 2016
Our DNA samples were within the range we believed it would be.

Molecular Cloning / Plasmid DNA Preparation – Training

June 8th, 2016
Project # 1
From Page # 1
To Page #14
Originally Recorded by: Brynne Schwabauer

Purpose: To perform Molecular Cloning

Materials:
1100uL of autoclave water & Molecular Cloning kit (Purple kit) & 100uL Lysis Buffer & 400uL Zippy Buffer & 300UL of neutralization buffer & 200uL Endotoxin Removing Buffer & Columns and Holders (4x)

Procedure:
1) Remove medium tube that contain bacteria created on 6/7/2016 from shaker (was in overnight)
2) Add to centrifuge (10min at 5000rmp)
3) Remove from centrifuge and dump out LB Broth into bleach (cells will remain in the bottom of the tube)
4) Check under UV light to see florescence (Still green)
5) Add 600uL of autoclave water to cells
6) Vortex the tube to mix and re-suspend the cells
7) Transfer suspended cells to eppendorf tubes
8) Use the solutions provided from the Molecular Cloning kit (Purple)
9) Add 100uL Lysis Buffer to tubes (Do not vortex)
10) Add 350uL of neutralization buffer to tubes
11) Centrifuge (5min at 21000rmp)
12) Set up Columns and Holders
13) Decant solution in Columns
14) Centrifuge (30 sec at 16000rmp)
15) Pour out Holder liquid
16) Add 200uL Endotoxin Removing Buffer
17) Centrifuge (30 sec at 16000rmp)
18) Add 400uL Zippy Buffer
19) Centrifuge (30sec at 16000rmp)
20) Centrifuge (2 min at 21000rmp) to remove ethanol
21) Remove from centrifuge and transfer to new eppendorf tubes
22) Add 30uL of autoclave water to each
23) Let sit for 1min to mix with DNA
24) Centrifuge (90 sec at 16000rmp)
25) Throw away column; keep eppendorf tubes with DNA
26) Nucleic Acid setting on NanoDrop 2000 machine
27) Add 2uL with water for a 'blanking' sample
28) Add 2uL of solution when measuring (See Data collected by the NanoDrop 2000 machine in Data below

Procedure for Cleaning Columns:

1) Wash Column with 750uL water
2) Spin down at 16,000 for 30seconds
3) Repeat Step 1
4) Wash Column with Fake Sample:
   -100uL of H2O
   -200uL of DNA Binding Buffer
5) Spin and Discard

Data: All data below was collected using the NanoDrop 2000 machine: (Step 25)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
1	1.72	1.22	129.3
2	1.63	0.85	201.2
3	1.82	2.2	102.1
4	1.67	0.98	212.2
	Desired: >1.8	Desired: 1.5-1.9	

Results: June 9th, 2016

We were able to see under UV light that our cells were no longer fluorescing.

Restriction Enzyme Digestion – Training

June 8th, 2016
Project # 1
From Page # 1
To Page #14
Originally Recorded by: Brynne Schwabauer

Purpose: To perform Restriction Enzyme Digestion

Materials:
2uL of NdeI & 14uL of test DNA & 2uL of SacI-HF & 2uL of Cutsmart & PCR tube

Procedure:

1) To prepare for the digestion, add all in PCR tube: 20uL total volume
   -14uL of test DNA (Used June 8th, 2016 NanoDrop samples 1 & 3, as they were the best (See Data June 8th, 2016))
   - 2uL of NdeI
   - 2uL of SacI-HF
   - 2uL of Cutsmart
2) Gently mix all together
3) Place in Thermocycler for 3hrs at 37degC
4) Remove and place in refrigerator at 4degC overnight

Molecular Cloning /DNA Purification by Electrophoresis / SLIC Cloning / Transformation – Training

June 9th, 2016
Project # 1
From Page # 1
To Page #14

Originally Recorded by: Josh Mueller, Therese Ninh & Brynne Schwabauer

Purpose: To perform Molecular Cloning and SLIC Cloning

Materials:
Materials used in Electrophoresis (6/7/2016) & Plating & CaCl DNA Recovery Kit (Yellow) & 5uL of 2.1 Buffer 1uL of T4 polymerate & 2.64 uL of PGFP-2 Vector (150ng) & 2uL 10x2.1Buffer & 0.26877uL Insert & 13.37uL Autoclaved Water & Genehog C.C. cells & 300uL LB Broth

Procedure:
1) Remove PCR tubes from refrigerator
2) Centrifuge samples (30 sec at 16000rmp)
3) Preform Electrophoresis on DNA sample
4) 1% of buffer volume (See DNA Purification by Electrophoresis: 6/7/2016)
   -Lane 1: DNA Ladder
   -Lane 3 & 4: GFP sample
   -Lane 6 & 7: GFP sample
   -Lane 9 & 10: m-Cherry sample
5) IF cutting out DNA pieces:
6) Remove gel and place sample under UV light (365 Wavelength)
7) Cut out gel: DNA Plasmid and Insertion part (m-Cherry)
8) Place cut pieces in eppendorf tubes
9) Take picture of gel without the DNA 'cut' sample: (Saved under '*6-9-2016AgroseGelpurificationofmCherryandpGFP1and2digestedwith NdeIandSacI')*
10) Purify the DNA through CaCl DNA Recovery Kit (Yellow)
11) Dilute 1uL of DNA samples with 1uL of water (Mathematically double concentration)
12) Add 2uL of diluted solution and test using NanoDrop 2000 machine (See data collected by the NanoDrop 2000 machine in Data below)
13) Calculate Cloning amounts (See Cloning Calculations in Data below)
14) Prepare diluted T4 polymerate by adding together in an eppendorf tube:
    - 5uL of 2.1 Buffer
    - 1uL of T4 polymerate

15) Prepare for Cloning by adding together in an Eppendorf tube (20uL total)
    -1uL of diluted T4 polymerate
    -2.64 uL of PGFP-2 Vector (150ng)
    -2uL 10x2.1Buffer
    -0.26877uL Insert
    -13.37uL Autoclaved Water
16) Gently mix together and place in Thermocycler (See Thermocycler Settings in Data below)
17) Remove Product from Thermocycler
18) Centrifuge sample
19) Add 4uL of product to Genehog C.C. cells (20uL prepared on 6/3/2016)
20) Stir gently (vigorous stirring may change C.C. cells)
21) Chill tubes for 20 minutes on ice
22) Heat tubes for 35sec at 42degC (Heat Shock)
23) Chill tubes for 2 minutes on ice (Immediately)
24) Add 300uL LB Broth in each
25) Add tubes to Shaker for 1hr (@37degC @250rpm)
26) Add 200uL of mixture on to one plate and 100uL on to another
27) Spread cells around plates using Bead technique
28) Remove beads from plate and place in bleach solution
29) Place plates in 37degC incubator

Data: Data below was collected using the NanoDrop 2000 machine: (Step 12)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Diluted Concentration (ng/uL)	Actual Concentration (ng/uL)
GFP-1 (Vector)	1.88	0.63	25.7	51.4
GFP-2	1.89	1.89	28.4	56.8
m-Cherry (Insert)	1.88	0.97	75.9	151.8
	Desired: >1.8	Desired: 1.5-1.9		

Data below is the Cloning Calculations used above: (Step 13)

- Mass of Insert and 150ng Vector needed:
  -150ng Vector: 5432bp & Insert: 737bp
  - Ratio Insert/Vector = I/V = 737bp/5432bp = 0.136
  - Vector Mass: 150ng *0.136 = 20.4ng (1:1 Ratio for Vector: Insert)
  - Insert Mass: 20.4ng *2 = 40.8ng (1:2 Ratio for Vector: Insert)
- Final amount based on Concentration:
  -Total Vector Amount Needed = Vector Mass *(1/Actual Concentration) = 150ng * (1/56.8ng/uL) = 2.64uL
  -Total Insert Amount Needed = Insert Mass *(1/Actual Concentration) = 40.8ng * (1/151.8ng/uL) = 0.26877uL

Data below is the settings used on the Thermocycler: (Step 16)

	Step 1	Step 2	Step 3	Step 4
DegC	22	75	37	4
Time	30 min	15 min	30 min	inf.

Molecular Cloning / DNA Sequencing – Training

June 13th, 2016
Project # 1
From Page # 1
To Page #14
Originally Recorded by: Brynne Schwabauer

Purpose: To perform Molecular Cloning and Plasmid DNA Isolation

Note: 10ng of Plasmid when Sequencing and 50ng of Plasmid when PCR-ing from Chromosomal DNA

Materials:
Materials used in Electrophoresis (6/7/2016) & Digestion (6/7/2016) & 0.75 uL Primer mix & Molecular Cloning kit (Purple) & 2.5 uL of KOD buffer & 1.5uL (25mm) of MgSO4 & 0.5uL of KOD Polymerase & 2.5uL of dNTPs & 16.75uL of Autoclave water & CaCl DNA Recovery Kit (Yellow) & 280ng/uL of DNA & 4.0 uL of 2um Primer (Either F or R, diluted from 100uM stock)

Procedure:
1) Perform Molecular Cloning kit (Purple) for the pmCherry Clones
2) Measure concentrations using Nanodrop (See Data collected by the NanoDrop 2000 machine in Data below)
3) Perform Digestion (See Digestion Calculations in Data below)
   - ❖ To prepare for the digestion, add all in an eppendorf tube:
     - ~uL DNA (Used June 13th, 2016 NanoDrop samples 1, 2 & 3, as they were the best (See Data June 13th, 2016))
     - 0.5uL of NdeI
     - 0.5uL of SbfI-HF
     - 1uL of Cutsmart
     - ~uL water (For a total volume of 10uL)
   - ❖ Gently mix all together
   - ❖ Place in Thermocycler for 3hrs at 37degC
4) Preform Electrophoresis on DNA and Digestion samples:
5) 1% of buffer volume (See DNA Purification by Electrophoresis: 6/7/2016)
   -Lane 1: DNA Ladder
   -Lane 2: Digestion 1 sample
   -Lane 3: Digestion 2 sample
   -Lane 4: Digestion 3 sample

-Lane 5: Digestion 4 sample
-Lane 7 & 8: DNA 1 sample
-Lane 10 & 11: DNA 2 sample
-Lane 13 & 14: DNA 3 sample

6) Perform Steps 5-10 of the Molecular Cloning /DNA Purification by Electrophoresis / SLIC Cloning / Transformation (6/9/2016)
7) Measure concentration of DNA Sample 1 using Nanodrop (See Data collected by the NanoDrop 2000 machine in Data below)
8) For PCR add together in a PCR tube:
   - 0.5uL of DNA
   - 0.75 uL Primer mix
   - 2.5 uL of KOD buffer
   - 1.5uL (25mm) of MgSO4
   - 0.5uL of KOD Polymerase
   - 2.5uL of dNTPs
   - 16.75uL of Autoclave water (For a total volume of 25 uL)
9) Gently mix the Eppendorf tube
10) Place in PCR machine; (See settings for PCR in Data below)
11) For sequencing add together in an eppendorf tube:
   - 280ng/uL of DNA in 8uL (See Sequencing calculations in Data below)
   - 4.0 uL of 2um Primer (For a total volume of 25 uL)
12) Place mixed samples in Sequencing tubes (# AFG401 & AFG402) and send in for sequencing

For Confirming a Clone Procedure:

1) Preform Miniprep on 35uL of DNA
2.1) Use 10uL of DNA to Digest DNA
2.2a) PCR a portion of DNA with sequencing primers (These primers may vary)
2.2b) Send PCR'ed sample in for sequencing in yellow tube
- 320ng of DNA (~8uL)
- 4uL of primer (2uM)

Data: Data below was collected using the NanoDrop 2000 machine: (Step 2)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
1	1.73	1.13	227.6
2	1.71	1.04	254.1
3	1.74	1.14	308.8
4	1.63	0.77	206.8
	Desired: >1.8	Desired: 1.5-1.9	

Data below is the Digestion Calculations used above: (Step 3)

- Mass of DNA sample needed 1ug = 1000ng
- DNA Sample A uL = 1000ng/Concentration A ng/uL
- Water Sample A = Total Volume – (Sample A uL + 2uL)
  - DNA Sample 1 = 1000ng/227.6 ng/uL = 4.39uL
  - Water Sample 1= 10uL – (4.39uL + 2uL) = 3.61uL
  - DNA Sample 2 = 1000ng/254.1 ng/uL = 3.93 uL

- Water Sample 2= 10uL – (3.93uL + 2uL) = 4.07uL
- DNA Sample 3 = 1000ng/308.8 ng/uL = 3.38 uL
- Water Sample 3 = 10uL – (3.38 uL + 2uL) = 4.762uL
- DNA Sample 4 = 1000ng/206.8 ng/uL = 4.83uL
- Water Sample 3= 10uL – (4.83uL + 2uL) = 3.16uL

Data below was collected using the NanoDrop 2000 machine: (Step 7)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
1	1.88	0.24	67.2

Desired: >1.8 Desired: 1.5-1.9

Data below is the settings used on the PCR: (Step 10)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	56	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	12sec	29 times	3 min	inf.

Data below is the Sequencing Calculations used above: (Step 11)

- Mass of DNA sample needed 280ng
- DNA Sample A uL = 280ng/Concentration A ng/uL
- Water Sample A = Total Volume – (Sample A uL)
  - DNA Sample 1 = 280ng/67.2 ng/uL = 4.167uL
  - Water Sample 1= 8uL – (4.167uL) = 3.83uL

Results: June 14th, 2016

Our sequencing results came in electronically, we performed it correctly.

DNA Competency Test – Training

June 14th, 2016
Project # 1
From Page # 1
To Page #14
Originally an iGEM test protocol
Recorded by: Brynne Schwabauer

Purpose: To perform DNA Competency test

Materials:
DNA from iGEM kit & 50 µL of competent cells & 200 µL of LB Broth

Procedure:

1) Centrifuge DNA: 30 sec at 10,000rmp (There should be 50 µL of DNA in each tube in iGEM Kit.)
2) Thaw competent cells on ice.
3) Label one 0.6 ml Eppendorf tube for each concentration and then pre-chill by placing the tubes on ice.
4) Add 1 µL of DNA into each eppendorf tube.
5) Add 40 µL of competent cells into each tube.
6) Incubate on ice for 20 -30 minutes
7) Heat tubes for 35sec at 42degC (Heat Shock)
8) Chill tubes for 2 minutes on ice (Immediately)
9) Add 200 µL of LB Broth per tube
10) Incubate at 37°C for 2 hours.
11) Label and warm agar plates in the mean time
12) Add 20 µL from each tube onto the appropriate plate, and spread the mixture evenly across the plate. (Do triplicates (3 each) of each tube if possible, so you can calculate an average colony yield.)
13) Incubate at 37°C overnight or approximately 16 hours.
14) Count the number of colonies.
2) If you've done triplicates of each sample, use the average cell colony count in the calculation

Data: Data below is how to calculate DNA concentration

- (colonies on plate) / ng of DNA plated x 1000ng/µg
- Note: The measurement "ng of DNA plated" refers to how much DNA was plated onto each agar plate, not the total amount of DNA used per transformation. You can calculate this number using the following equation:
- 1 µL x concentration of DNA (refer to vial) x (volume plated / total reaction volume)

Results: June 15th, 2016

- In this first time we only plated a total of 5 plates: One for each concentration

DNA Concentration Guidelines:

DNA concentration	0.5pg/ul	5pg/ul	10pg/ul	20pg/ul	50pg/ul
# of colonies	10 - 20	120 - 170	280 - 360	480 - 802	500 - 1000+

June 15, 2016 – Creating New Competent Cells

June 15th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Preparing new Competent Cells

Materials:

- Materials used to Competent Cells (6/3/2016)

Procedure:

1) Created new Chemical Competent Cells (See E-Coli Chemical Bacteria Cells in Liquid Media / Competent Cells Preparation: 6/3/2016)

Results:

We created 44 eppendorf tubes (40uL each) full of competent cells; we will need to test how well they perform later.

Rehydrate PYeaR -GFP DNA
Transformation of Genehog Cells

June 16th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: To transform Genehog cells with PYeaR-GFP

Notes: We will be transforming our Genehog cells (Created June 3rd 2016)

Materials:
Materials needed for Transformation (6/62016) & 10uL of autoclave water &
GFP DNA from the iGEM kit(Plate 1, Well 4B)

Procedure:

1. Transform competent cells created on June 15th, 2016 (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)
2. We will be using the GFP DNA from the iGEM kit: Plate 1, Well 4B (If Nitrate is present then it should fluoresce green)
3. To rehydrate GFP DNA from the iGEM kit: Plate 1, Well 4B
   -Puncture DNA well
   -Add 10uL of autoclave water
   -Mix by pipetting up and down
   -Allow to sit for a minute
   -Transfer to an eppendorf and place in 4degC freezer
4. Mix well and Streak
5. Incubate in 37degC overnight

Results:
They grew well in their plates. We were able to successfully inoculate on June 19th, 2016.

Inoculation of PYeaR-GFP DNA_

June 19th, 2016

Project # 2
From Page # 15
To Page #
Originally Recorded by: Danny Dooling

Purpose: Inoculating PYeaR-GFP

Materials:
Materials used in Inoculation & 5 mL of LB Broth & 5 uL of CM34

Notes: This test is called (PYeaR-GFP Set A)

Procedure:
1) Inoculate colony from pYeaR plate into
   - 5 mL of LB Broth
   - 5 uL of CM34 [1:1000])
2) Repeat step 1 with different colonies to attain 3 different culture tubes.
3) Place in shaker overnight (approx. 16 hours) (@37degC @250rpm)

Results: We will see the results on June 21st, 2016

2nd Inoculation of PYeaR-GFP DNA _

June 20th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Danny Dooling

Purpose: 2nd inoculation of PYeaR-GFP DNA and testing Nitrate Sensor

Notes: For every 1ug of DNA, how many theoretical colonies should grow? Must be less than 5,000 colonies…. around 1,000 colonies is good.

Materials:
Cell culture & 50uLof 1.0M KNO_3 & 250 uL of 0.1 M KNO_3

Procedure:
1) Remove tubes from shaker
2) To three eppendorf tubes:
   -Add 50 uL of cell culture
   - 5 mL LB broth
   - 5 uL of cm34 [1:1000]
3) Repeat step 2 with each cell culture tube. (Creating a total of 9 tubes)
4) Place in shaker for 2 hours (@37degC @250rpm)
5) Keep in shaker until OD_{600} is measured between 0.4-0.6 (See OD_{600} Data in Data below)
   -Using Well technique not Cuvette technique

6) Calculate Transformation Efficiency (See Transformation Efficiency Calculations in Data below)
7) Once desired OD reading is reached
   -Add 50 uL of 0.1 M KNO_3 into one tube
   -Add 250 uL of 0.1 M KNO_3 into another tube. (To create 3 cultures with 0 mM , 1 mM , and 5 mM KNO_3)
8) Place 9 tubes (3 of each concentration) into shaker overnight (@37degC @250rpm)
9) Preform inoculation (the exact same from June 19th, 2016) this time only using two separate colonies (PYeaR-GFP Set B)

Results: Data below was collected using the OD_{600} machine: (Step 5)

DNA Concentration	0.5 pg/uL (Outlier)	5 pg/uL	10 pg/uL	20 pg/uL	50 pg/uL
# of Colonies	0	8	10	49	430
Transformation Efficieny	0	1.92*10^7 cfu/ug	1.2*10^7 cfu/ug	2.94*10^7 cfu/ug	1.032*10^8 cfu/ug

Transformation Efficiency Average (Outlier not included) : 4.095*10^7 cfu/ug

Data below is the Transformation Efficiency Calculations used above: (Step 6)

- Transformation Efficiency (cfu/ug) = [# of Colonies / (ug DNA *Dilution Factor)]

Testing PYeaR-GFP DNA with Phosphorus Buffer / Plating MG1655

June 21st, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Danny Dooling

Purpose: Testing PYEAR - GFP DNA with Phosphorus Buffer and Pellet

Notes: Excitation Max is 501 nm and Emission Max 511nm wavelength. This is a bit of a problem as they need

Materials:
120uL of water & 1 ml of cell culture PYeaR-GFP Set A & 1uL of CM 34 & 1ml of LB Broth

Procedure: PYeaR-GFP Set A

1) To Create 10x Phosphate Buffered Saline (originally from PBS): To 500ml of Water
   - 50g of NaCl
   - 1.25g of KCl
   - 9.0g of Na_2HPO_4
   -1.5g of KH_2PO_4
- Adjust pH to 7.4

- ❖ Adjust volume to 1 L with additional distilled H2O

2) Sterilize Buffer by autoclaving.
3) Remove all tubes from shaker
4) Add 1 ml of cell culture PYeaR-GFP Set A in an eppendorf tube
5) Spin down for 2 minutes at 21000rpm: remove supernatant broth.
6) Suspend pellet in 1 ml of phosphate buffer
7) Spin down for 2 min at 21000 to pellet
8) Suspend cells in 1 ml of phosphate buffer again
9) In the transparent well plate (For overnight cell culture)
   -Add 30uL of suspended cells
   -Add 120uL of water (Final Volume 150uL)
10) In Black well plate (For suspended cells)
   -Add 150 uL of suspended cells
11) Spin down eppendorf tubes to pellet, discard supernatant phosphate buffer.
12) Observe dry pellets on UV plate
13) Plate MG1655 for tomorrow (See June 6th, 2016) Do not use CM34, as MG1655 is not antibiotic resistant
14) Place in 37deg incubator overnight

Note: If this works then proceed to Well Plate Procedure, if not repeat procedures starting form June 19th, 2016

Large Well Plate: Testing Nitrate Sensor at Different Concentrations

June 21st, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Danny Dooling

Purpose: To create multiple data points to see how precise our Nitrate sensor is

Material:
50g of NaCl & 1.25g of KCl & 9.0g of Na_2HPO_4 & 1.5g of KH_2PO_4 &
10uL of cell culture PYeaR-GFP Set B

Well Plate Procedure: PYeaR-GFP Set B

1) In Well Plate (in 46 of the 96 wells) add:
   -Add 1ul of CM 34
   -1ml of LB Broth
   -Add 10 uL PYeaR-GFP Set B (1B into 23 wells and the 2B into the other half)
2) Place in Shaker for 2 - 2.5 Hours
3) Keep in shaker until OD_{600} is measured between 0.4-0.6

4) Add corresponding Nitrate concentrations (See Nitrate Concentrations in Data below)
5) Place in 37degC shaker overnight

Results: See Data June 22nd, 2016
Overall this experiment had relative success. The lower nitrate concentration graphs (below) were all over the place. Though, as we moved into the greater nitrate concentrations, the ODs became much more linear like expected. With these results we can say that the nitrate sensor is not very precise when detecting such low levels of nitrate. This is not too surprising as the previous team only tested their sensor between 2mM and 10 mM.(We tested between 0.00025 mM and 1 mM).

Data: Data below was used for Nitrate Concentrations: (Step 4)

Stock Volume (L)	Stock Concen (M)	mass KNO3 (g)	Volume Innoculated (mL)	Innoc. Concen (mM)	LB Added			Volume Stock added (uL)	Colony 1	C
0.25	0.001	0	0.85	0.00025	0.8497875	0.000249938	0.024993752	0.2125	A2	
0.25	0.001	0	0.85	0.0005	0.849575	0.00049975	0.049975012	0.425	A3	
0.25	0.001	0	0.85	0.00075	0.8493625	0.000749438	0.074943792	0.6375	A4	
0.25	0.001	0	0.85	0.001	0.84915	0.000999001	0.0999001	0.85	A5	
0.5	0.001	0	0.85	0.00125	0.8489375	0.001248439	0.124843945	1.0625	A6	
0.5	0.001	0	0.85	0.0015	0.848725	0.001497753	0.149775337	1.275	A7	
0.5	0.001	0	0.85	0.00175	0.8485125	0.001746943	0.174694285	1.4875	A8	
0.5	0.001	0	0.85	0.002	0.8483	0.001996008	0.199600798	1.7	A9	
0.5	0.001	0	0.85	0.004	0.8466	0.003984064	0.398406375	3.4	A10	
0.5	0.001	0	0.85	0.006	0.8449	0.005964215	0.596421471	5.1	B1	
0.5	0.001	0	0.85	0.008	0.8432	0.007936508	0.793650794	6.8	B2	
0.25	0.001	0	0.85	0.01	0.8415	0.00990099	0.99009901	8.5	B3	

0.1	0.85	0.02	0.84983	0.019996001	0.019996001	0.17	B4
0.1	0.85	0.04	0.84966	0.039984006	0.039984006	0.34	B5
0.1	0.85	0.06	0.84949	0.059964022	0.059964022	0.51	B6
0.1	0.85	0.08	0.84932	0.079936051	0.079936051	0.68	B7
0.1	0.85	0.1	0.84915	0.0999001	0.0999001	0.85	B8
0.1	0.85	0.2	0.8483	0.199600798	0.199600798	1.7	B9
0.1	0.85	0.4	0.8466	0.398406375	0.398406375	3.4	B10
0.1	0.85	0.6	0.8449	0.596421471	0.596421471	5.1	C1
0.1	0.85	0.8	0.8432	0.793650794	0.793650794	6.8	C2
0.1	0.85	1	0.8415	0.99009901	0.99009901	8.5	C3

Measuring GFP Expression / Inoculating MG1655

June 22nd, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Measuring GFP Expression and Inoculating MG1655

Note: MG 1655 is a strain of *E Coli* and it eats glucose and grows. We will be extracting the NAP genes from it.

Materials:
Materials used for Inoculation & 120uL water & 1 ml of phosphate buffer (created June 21st, 2016) & Plated samples of MG 1655

Procedure:

1) Remove well from shaker
2) Spin down for 10 minutes at 5000rpm: remove supernatant broth.
3) Suspend pellet in 1 ml of phosphate buffer
4) Spin down for 10 min at 5000 to pellet
5) Suspend pellet in matched volume of phosphate (0.85ml)
6) Add 30 uL from each well into a corresponding clear well,
   -Add 120uL water to each well
7) Add 150uL from each well into a corresponding new black well
8) Measure GFP expression
9) Inoculate MG1655 (See June 19th, 2016) Do not use CM34, as MG1655 is not antibiotic resistant

Results:
We were able to successfully inoculate MG1655 and will use it in the next few steps of our experiment.

Final OD for Nitrate Concentrations:

Final OD	1	2	3	4	5	6	7	8	9	10	11	12	
A													Corrected [Blank 600]
B	0.61	0.659	0.582	0.829	0.594	0.63	0.739	0.803	0.658	0.668			Corrected [Blank 600]
C	0.574	0.576	0.551	0.562	0.654	0.681	0.485	0.556	0.557	0.532			Corrected [Blank 600]
D	0.588	0.532	0.348	0.153	0.381	0.423	0.464	0.673	0.863	0.331			Corrected [Blank 600]
E	0.594	0.714	0.679	0.814	0.757	0.781	0.663	0.677	0.588	2.587			Corrected [Blank 600]
F	0.643	0.623	0.698	0.672	0.689	0.683	0						Corrected [Blank 600]

Final Florescence for Nitrate Concentrations:

Glow	1	2	3	4	5	6	7	8	9	10	11	12	
A													Blank 504,534
B	1207	1230	1299	1199	1214	1203	1226	1314	1249	1254			Blank 504,534
C	1232	1164	1096	1079	1123	992	1244	1137	2193	2477			Blank 504,534
D	2443	2826	4310	533	810	781	943	1085	1220	717			Blank 504,534
E	1482	1280	1254	1181	1340	1025	1234	1112	1330	2188			Blank 504,534
F	1553	1717	2162	2447	3252	3704	0						Blank 504,534

Calculated Relative Florescence Unit (RFU):
- RFU = Florescence /(OD*5)
-We multiplied OD by 5, as the ratio is 30:150

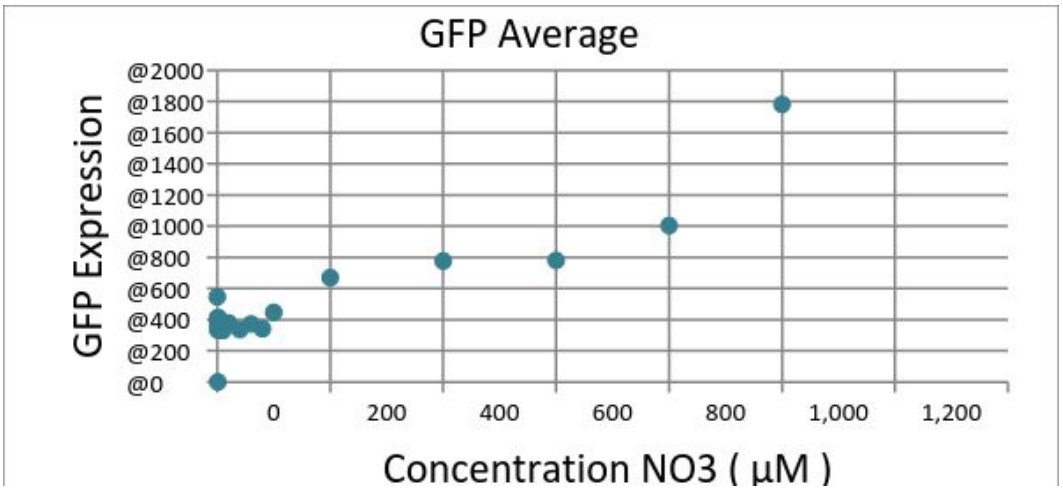
RFU	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	395.74	373.29	446.39	289.26	408.75	381.90	331.80	327.27	379.64	375.45		
C	429.27	404.17	397.82	383.99	343.43	291.34	512.99	408.99	787.43	931.20		
D	830.95	1062.41	2477.01	696.73	425.20	369.27	406.47	322.44	282.73	433.23		
E	498.99	358.54	369.37	290.17	354.03	262.48	372.25	328.51	452.38	169.15		
F	483.05	551.20	619.48	728.27	943.98	1084.63						

Final Concentrations for each well (See graphs below):

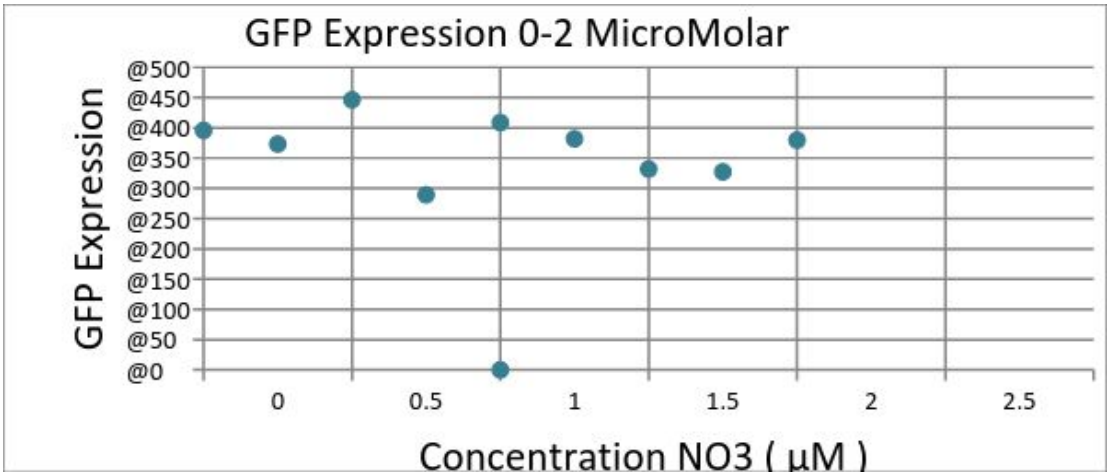
Conc (mM)	Conc (uM)	Colony 1	Colony 2	Average

0	0	395.74	696.73	546.2348655
0.00025	0.25	373.29	425.20	399.2448592
0.0005	0.5	446.39	369.27	407.829446
0.00075	0.75	289.26	406.47	347.8648455
0.001	1	408.75	322.44	365.5955293
0.00125	1.25	381.90	282.73	332.3197042
0.0015	1.5	331.80	433.23	382.5161789
0.00175	1.75	327.27	498.99	413.1313131
0.002	2	379.64	358.54	369.0893379
0.004	4	375.45	369.37	372.4079088
0.006	6	429.27	290.17	359.7201414
0.008	8	404.17	354.03	379.0978644
0.01	10	397.82	262.48	330.1530682
0.02	20	383.99	372.25	378.1165628
0.04	40	343.43	328.51	335.9666003
0.06	60	291.34	452.38	371.8586113
0.08	80	512.99	169.15	341.0715752
0.1	100	408.99	483.05	446.0205086
0.2	200	787.43	551.20	669.3182637
0.4	400	931.20	619.48	775.3436241
0.6	600	830.95	728.27	779.6130952
0.8	800	1062.41	943.98	1003.191396
1	1000	2477.01	1084.63	1780.819071

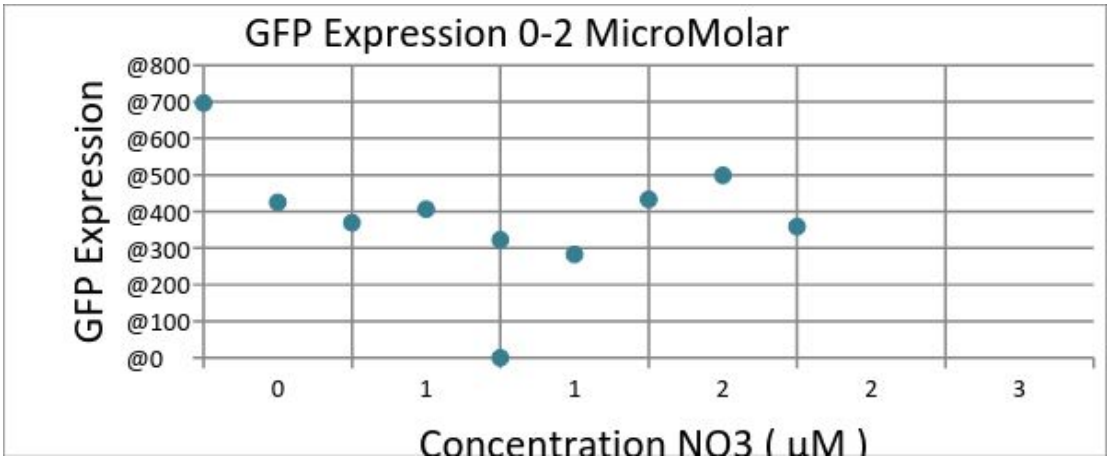
Total Colony Average: GFP Expression



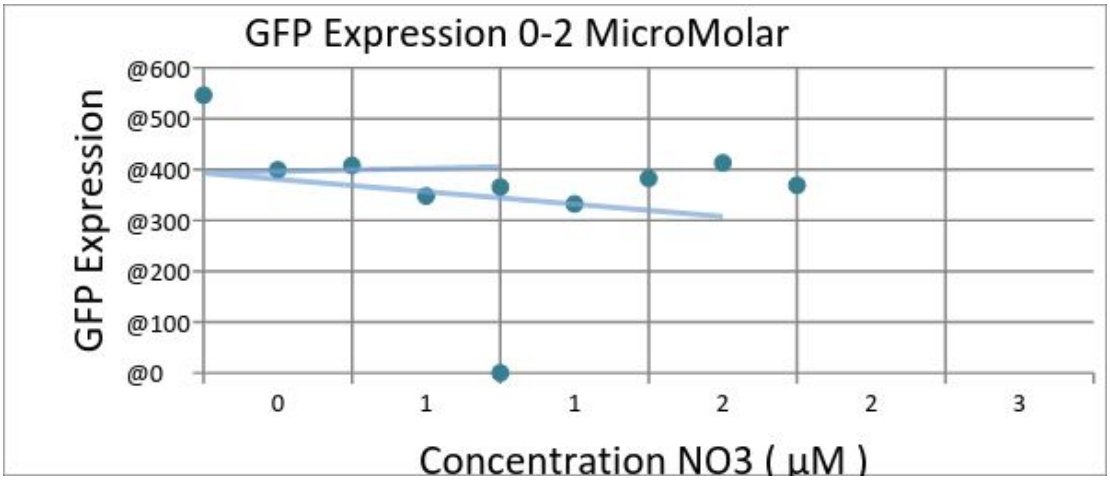
COLONY 1: GFP Expression 0 – 2 MircoMolar



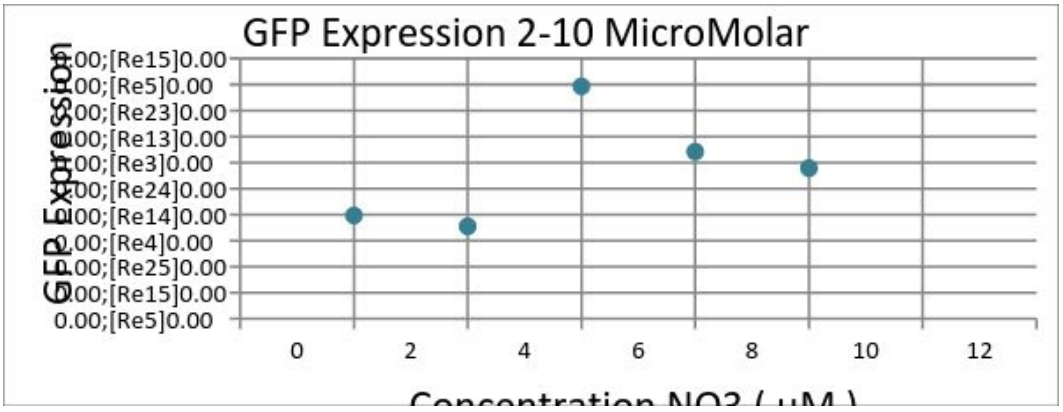
COLONY 2: GFP Expression 0 – 2 MircoMolar



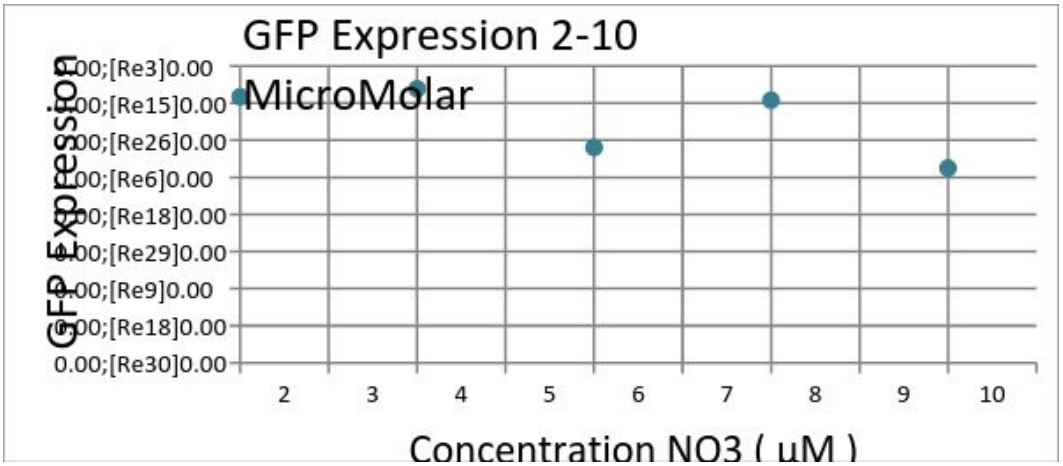
COLONY Average: GFP Expression 0 – 2 MircoMolar



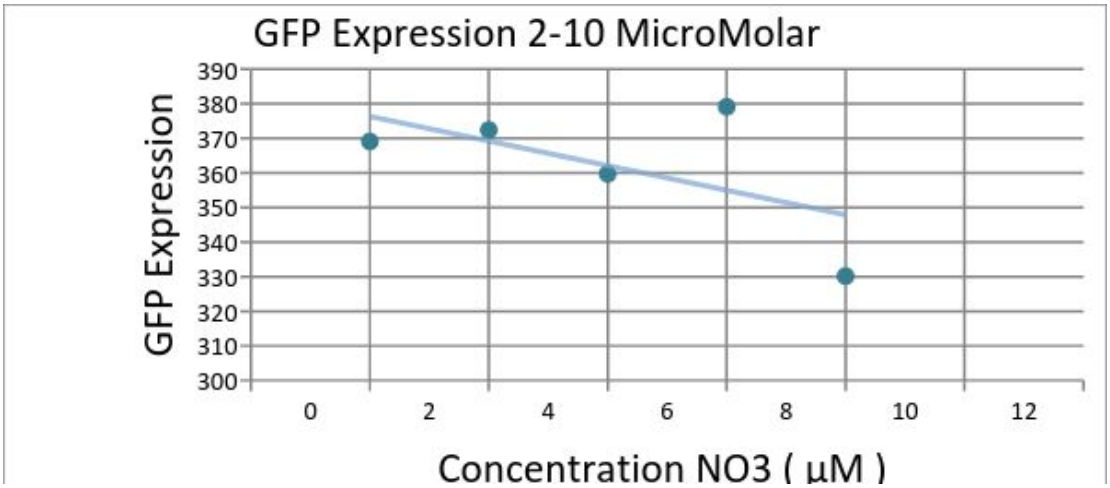
COLONY 1: GFP Expression 2 – 10 MircoMolar



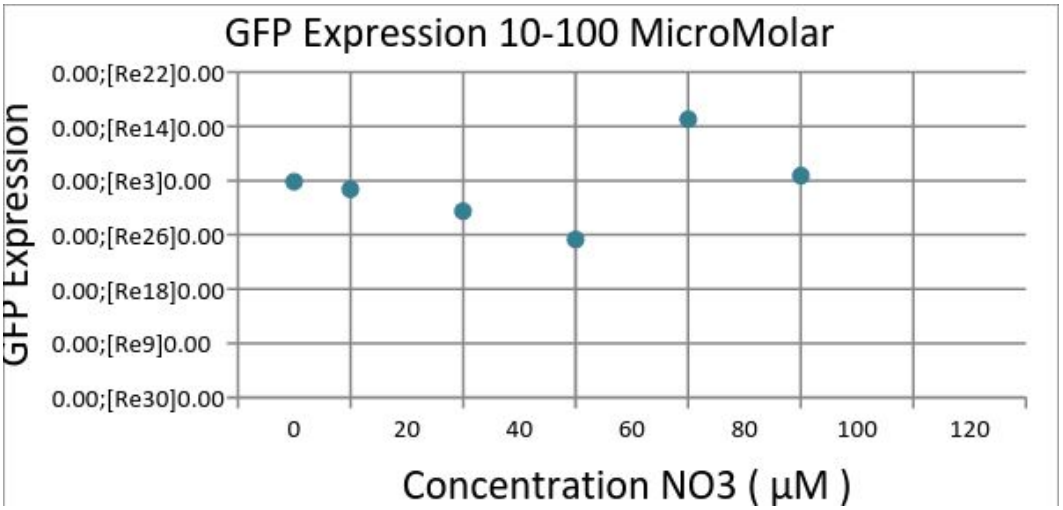
COLONY 2: GFP Expression 2 – 10 MircoMolar



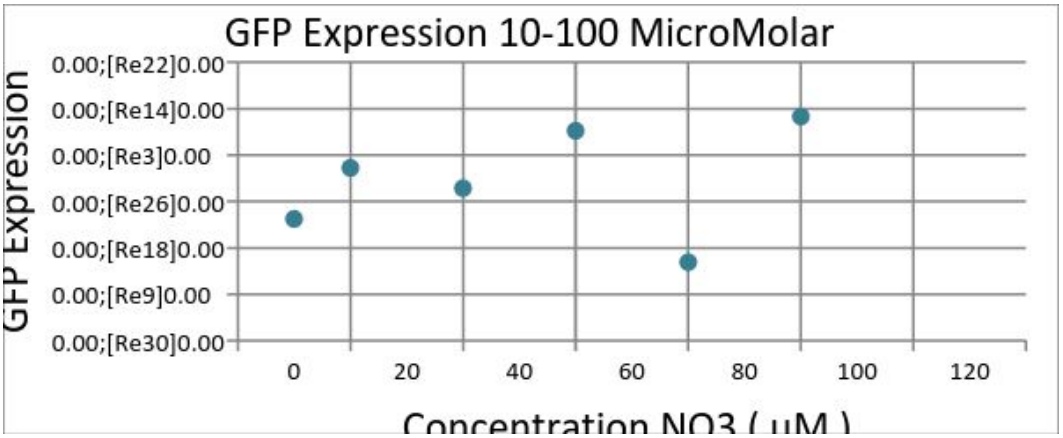
COLONY Average: GFP Expression 2 – 10 MircoMolar



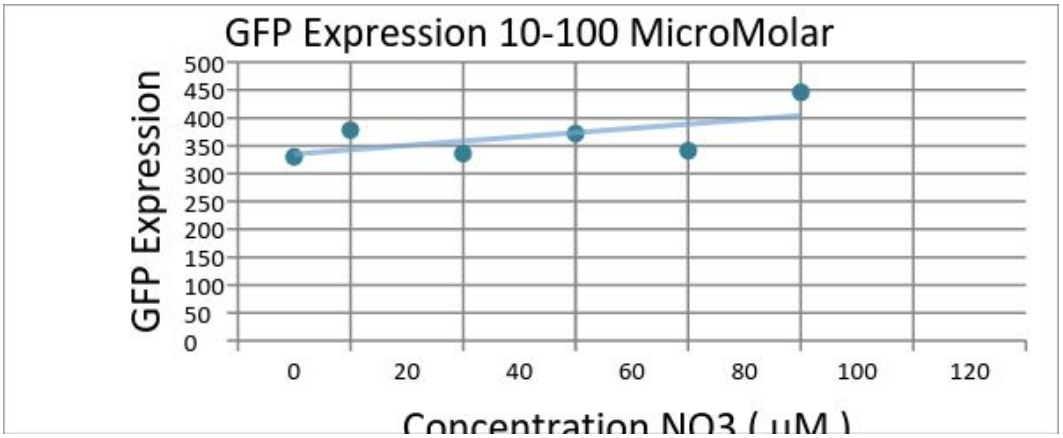
COLONY 1: GFP Expression 10 – 100 MircoMolar



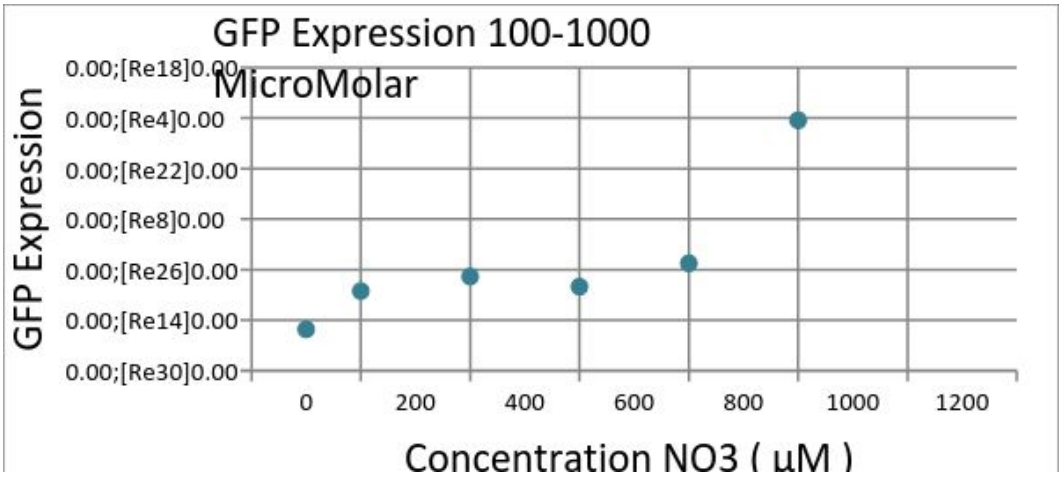
COLONY 2: GFP Expression 10 – 100 MircoMolar



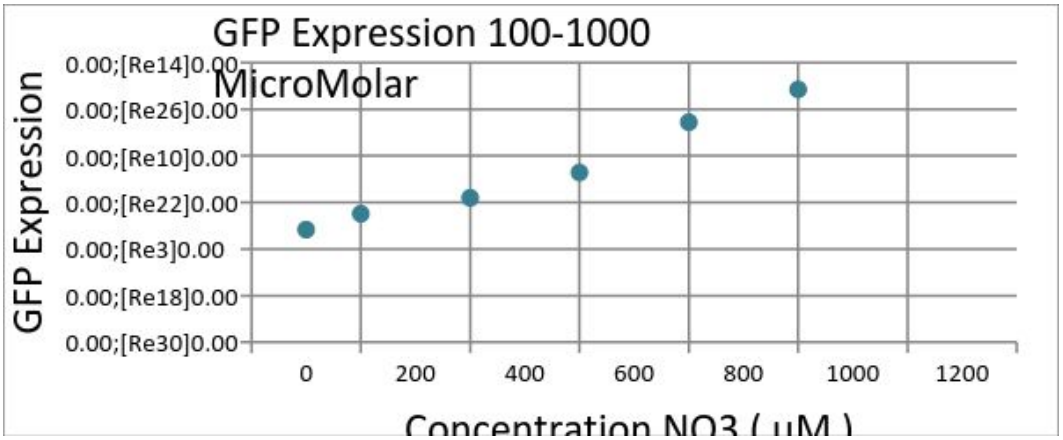
COLONY Average: GFP Expression 10 – 100 MircoMolar



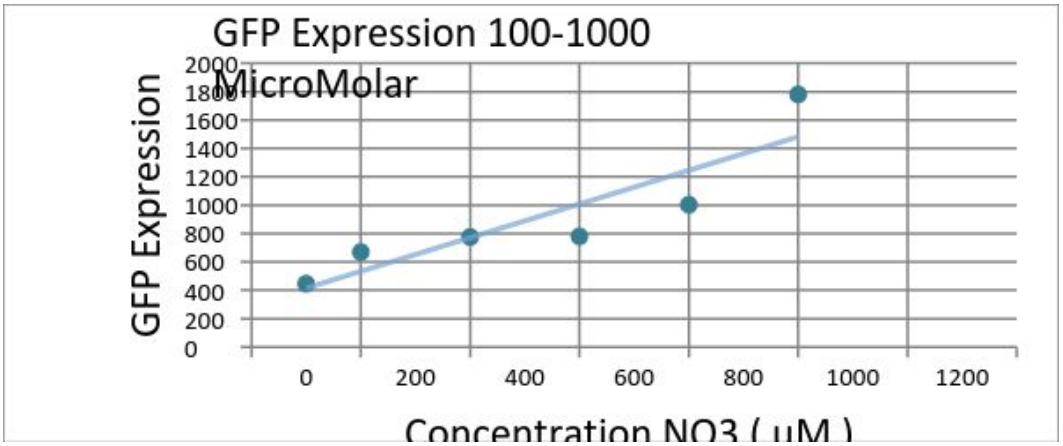
COLONY 1: GFP Expression 100 – 1000 MircoMolar



COLONY 2: GFP Expression 100 – 1000 MircoMolar



COLONY Average: GFP Expression 100 – 1000 MircoMolar



Gram Negative Bacterial Cell Lysate

June 23rd, 2016
Project # 1
From Page # 15
To Page #
Originally emailed by Erome procedure
Recorded by: Brynne Schwabauer

Purpose: To prepare Gram Negative Bacterial Cell Lysate

Notes: We created tubes 1 & 2 and then eluted (See Eluting DNA: 6/23/2016) them to A1 and A2 respectively. We now have 4 samples: 1, 2, & A1, A2

Materials:
180uL of PureLink Genomic Digestion Buffer & 20uL of Proteinase K & 20uL RNase A &
200uL PureLink Genomic Lysis/Digestion Buffer & 200uL 96 - 100% of ethanol &
500 µL Wash Buffer 1 & 500 µL Wash Buffer 2

Procedure:

1) Set a water bath or heat bath to 55degC
2) Harvest up to 2 x 109 Gram negative (~1mL of overnight *E. Coli* culture by centrifugation - 10 minutes at 5000rmp)
3) Suspend the cell pellet in 180uL of PureLink Genomic Digestion Buffer
4) Add 20uL of Proteinase K (In supplied Kit) to lyse the cells
5) Vortex briefly
6) Incubate for 30min – 4 hours at 55degC, with occasional vortexing, until lysis is complete
   -Begin: 12:48pm, End: 1:15pm
7) Add 20uL RNase A (In supply Kit) to lystate
8) Mix well and vortex briefly, incubate at room temperature for 2 minutes
9) Add 200uL PureLink Genomic Lysis/Digestion Buffer
10) Mix well and vortex briefly to obtain a homogeneous buffer
11) Add 200uL 96 - 100% of ethanol to the lysate
12) Mix well and vortex for 5 seconds to obtain a homogenous buffer
13) To Bind DNA:
    - Remove a PureLink Spin Column in a Collection Tube from the package.
    - Add the lysate (~640 µL) prepared with PureLink Genomic Lysis/Binding Buffer and ethanol to the PureLink® Spin Column.
    - Centrifuge the column at 10,000 × g for 1 minute at room temperature.
    -Note: If you are processing >200 µL starting material such as blood, buccal swabs, or Oragene™ preserved saliva, you need to perform multiple loading of the lysate by transferring any remaining lysate to the same PureLink® Spin Column (above) and centrifuge at 10,000 × g for 1 minute.
    - Discard the collection tube and place the spin column into a clean PureLink® Collection Tube supplied with the kit.
14) To Wash DNA:
    -Add 500 µL Wash Buffer 1 prepared with ethanol (page 23) to the column.
    -Centrifuge column at room temperature at 10,000 × g for 1 minute.
    -Discard the collection tube and place the spin column into a clean PureLink® collection tube supplied with the kit.
    -Add 500 µL Wash Buffer 2 prepared with ethanol (page 23) to the column.
    -Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.

Eluting DNA

June 23rd, 2016
Project # 1
From Page # 15
To Page #
Originally emailed by Erome procedure
Recorded by: Brynne Schwabauer

Purpose: Eluting DNA

Notes: We created tubes 1 & 2 (See Gram Negative Bacterial Cell Lysate: 6/23/2016) and then eluted them to A1 and A2 respectively. At the end of this procedure we will have 4 samples: 1, 2, & A1, A2 as of June 23rd, 2016

Materials:
50 µL of PureLink Genomic Elution Buffer & Sample 1 (6/23/2016) & Sample 2 (6/23/2016)

Procedure:

1) Place the spin column in a sterile 1.5-mL microcentrifuge tube.
2) Add 25–200 µL of PureLink Genomic Elution Buffer to the column.
3) Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature. The tube contains purified genomic DNA.
4) To recover more DNA, perform a second elution step using the same elution buffer volume as first elution in another sterile, 1.5-mL microcentrifuge tube.
5) Centrifuge the column at maximum speed for 1.5 minutes at room temperature. The tube contains purified DNA. Remove and discard the column. Storing DNA
6) Store the purified DNA at –20°C or use DNA for the desired downstream application.
   - For long-term storage, store the purified DNA in PureLink Genomic Elution Buffer at –20°C as DNA stored in water is subject to acid hydrolysis. -To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.

M9 Glucose/Leucine Minimal Medium

June 27th, 2016
Project # 1
From Page # 15
To Page #
Originally emailed by Erome procedure
Recorded by: Brynne Schwabauer

Purpose: Preparation of Leucine Medium

Note: We also Bleached and Autoclaved our used glassware

Materials:
M9 Salts 5x & 30.0 g/L Na2HPO4 & 15.0 g/L KH2PO4 & 5.0 g/L NaH4Cl & 2.5 g/L NaCl

Procedure:

1) *To make 20% (w/v) glucose:*
   -Add 10 g of glucose to 47 mL of DI water and autoclave.
   -Store at room temperature
2) *To make 1 M $MgSO_4$ dissolve:*
   -Add 24.65 g of $MgSO_4 7H_2O$ (m.w. 246.47) in DI water
   -Adjust final volume to 100 mL, then autoclave
   -Store at room temperature
3) *To make 500 mM $CaCl_2$:*
   -Dissolve 7.35 g of $CaCl_2$ $2H_2O$ (m.w. 147.01) in DI water
   -Adjust the final volume to 100 mL, then autoclave.
   -Store at room temperature
4) *To make thiamine (10 mg/mL) stock solution:*
   -Dissolve 100 mg of thiamine in 10 mL of sterile DI water.
   - Further sterilize the stock solution by passage through 0.2 µ pre-sterilized filter
   - Store at 4 °C in foil-wrapped tube.
5) *To make 30 mM leucine (m.w. 131.17) stock solution:*
   -Dissolve 0.039 g leucine in 10 mL of DI water.
   -Further sterilize the stock by passage through 0.2 µ pre-sterilized filter
   -Store at 4 °C freezer

Data: Volumes and Concentrations of Leucine Medium

	M9 Salts	MgSO4 (1M)	CaCl2 (500nM)	Thiamine (10mg/mL)	Leucine (30mM)	Antibiotic 20% w/v Glucose	Stock Sterile Water
Final Volume (100ml)	20 mL	0.1 mL	0.05 mL	0.1 mL	1.0 mL	5.0 mL	73.0 mL
Final Concentration	1x	1 mM	0.25 mM	10.0 mM	0.3 mM	Total:	1%

Results:

The cells in the minimal medium did not grow.

NapBC Primer/ Inoculate PYear GFP & SerA and Plated NirS

June 28th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Josh Mueller

Purpose: Preparation of NapBC Primer, inoculation of PYeaR GFP & SerA and Plating of NirS

Notes:

- We created tubes 1 & 2 (See Gram Negative Bacterial Cell Lysate: 6/23/2016) and then eluted (See_Eluting DNA: 6/23/2016) them to A1 and A2 respectively. We now have 4 samples: 1, 2, & A1, A2
- We used Sample 1 for this experiment as it had the better concentration (See Data collected by the NanoDrop 2000 machine in Data below)

Materials:

Materials for PCR (6/7/2016) & Electrophoresis (6/7/2016) & Inoculation & - Sample 1 (Created 6/23/2016) & Sample A1 (Created 6/23/2016) & Samples of NirS & Samples of PYeaR GFP & Samples of SerA bacteria & Kanamycin (KAN)

Procedure:

1) Measure concentration of DNA Sample 1 and Sample A1 using Nanodrop (See Data collected by the NanoDrop 2000 machine in Data below)
2) Prep NapBC Primer (See PCR: To Create SLIC sample - Step 1: 6/7/2016)
3) Dilute Sample 1 to 1/5 using autoclave water
4) Perform 1st PCR Run on 50ng of Sample 1 (See settings for 1st PCR Run in Data below)
5) In the mean time prepare 1% Electrophoresis Gel (See DNA purification by Electrophoresis: 6/7/2016)
6) Perform 2nd PCR Run on the same 50ng of Sample 1 (See settings for 2nd PCR Run in Data below)
7) Inoculate PYeaR GFP in Minimal Media
8) Inoculate SerA bacteria in LB Broth
9) Plated NirS in Agar + Kanamycin (KAN)
10) Cultured Jw2880 ∆ SerA + Kanamycin (KAN)

Data:

Data below was collected using the NanoDrop 2000 machine: (Step 1)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
Sample 1	1.78	1.59	481.2
Sample A1	1.8	1.78	627
	Desired: >1.8	Desired: 1.5-1.9	

Data below is the settings used on the 1st PCR Run: (Step 4)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	56	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	12 sec	29 times	3 min	inf.

Data below is the settings used on the 2nd PCR Run: (Step 6)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	56	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	43 sec	29 times	3 min	inf.

Results: We accidentally used the wrong type of clean media and had to redo part of the experiment. June 29th, 2016 Our second round of clean media is working-ish.

Glycerol Stock

June 29th, 2016
Project # 2
From Page # 15
To Page #
Originally emailed by Erome procedure
Recorded by: Josh Mueller

Purpose: Preparation of Glycerol Stock

Notes: Perform Glycerol Stock in Sterile Environment

Materials:
500ml of 50% Glycerol stock & 750 uL of overnight culture

Procedure:
1) Obtain two 2ml screw top tubes with 500ml of 50% Glycerol stock (This is already pre-prepared)
2) Add 750 uL of overnight culture to Glycerol stock tube (We used our Jw2880 ∆SerA + KAN: Cultured 6/27/2016)
3) Vortex mixture
4) Store in -80degC fridge (In our Genehog box)

Electrophoresis and Purification of NapA & NapBC

June 29th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer & Therese Ninh & Danny Dooling

Purpose: Electrophoresis and purification of NapA and NapBC

Note: All of our minimal media going forward will contain 8.5uM of NaCl and 5uM of FeSO4 to help cell growth, unless specified otherwise.

Materials:
Materials for Electrophoresis & Minimal Media & NapA Sample & NapBC Sample &
CaCl DNA Recovery Kit (Yellow)

Procedure:
1) Preform Electrophoresis on NapA and NapBC Primers:
2) Use 1% of buffer created June 28th, 2016 (See DNA Purification by Electrophoresis: 6/7/2016)
   -Lane 1: DNA Ladder
   -Lane 3 & 4: NapA sample
   -Lane 6 & 7: NapBC sample
3) Perform Steps 5-12 of the Molecular Cloning /DNA Purification by Electrophoresis / SLIC Cloning / Transformation (6/9/2016)
   - Do step 3 on NapBC only: as NapA did not perform as desired in the electrophoresis
     -See Data collected by the NanoDrop 2000 machine in Data below
4) Place NapBC in 4degC fridge
5) For NapA: throw away electrophoresis gel
   - We will start over with NapA, PCR using gradient temperatures and then electrophorese the samples again.
6) Prepare five samples of 10 uL total volume mixtures in PCR tubes using NapA
   - For this procedure, double the amounts in Step 2 of the PCR procedure (See PCR: 6/7/2016)
   - Create a large batch in a separate tube then aliquot it into the separate PCR tubes
7) Place in Thermocycler (See settings for PCR & Gradient Temperatures in Data below)
8) Prepare 1% Electrophoresis Gel for NapA (See DNA purification by Electrophoresis: 6/7/2016)
9) Perform Electrophoresis on NapA samples:
   -Lane 1: DNA Ladder

-Lane 2: Sample 1 NapA
-Lane 4: Sample 2 NapA
-Lane 6: Sample 3 NapA
-Lane 8: Sample 4 NapA
-Lane 10: Sample 5 NapA

10) Now perform Steps 5-12 of the Molecular Cloning /DNA Purification by Electrophoresis / SLIC Cloning / Transformation (6/9/2016) on NapA
    -See Data collected by the NanoDrop 2000 machine in Data below
11) Place NapA in 4degC freezer
12) Take the inoculated PYeaR(A) in Minimal Media (inoculated on 6/28/2016) and subculture that into new Minimal Media(B)
13) Place both the 1st Inoculated PYear(A) and the 2nd sub cultured PYeaR(B) into the 37degC shaker overnight.

Data:

Mass of NapBC gel is 0.216g
Mass of NapA gel is 0.155g

Data below was collected using the NanoDrop 2000 machine: (Step 4)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Diluted Concentration (ng/uL)	Actual Concentration (ng/uL)
NapBC	1.84	1.73	36.6	73.2
	Desired: >1.8	Desired: 1.5-1.9		

Data below is the settings used on the PCR: (Step 8)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	Gradient Temps.	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	49 sec	29 times	3 min	inf.

Step 3 Gradient Temperatures:
Sample 1 NapA: 66 degC
Sample 2 NapA: 64 degC
Sample 3 NapA: 62.1 degC
Sample 4 NapA: 59.8 degC
Sample 5 NapA: 57.9 degC

Data below was collected using the NanoDrop 2000 machine: (Step 10)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Diluted Concentration (ng/uL)	Actual Concentration (ng/uL)
NapA	1.96	0.28	17.6	35.2
	Desired: >1.8	Desired: 1.5-1.9		

Creating CM34, KAN, AMP, Tetra Plates & Subculturing PYeaR (B) _

June 30th, 2016
Project # 2
From Page # 15
To Page #

Originally Recorded by: Brynne Schwabauer

Purpose: Preparing new Plates and Subculturing PYeaR(B)

Notes: Our samples were not reaching OD in Step 6. Professor Guo told us to add the nitrates regardless. We will see our results on July 7th, 2016

Materials:

Materials for plating & Kanamycin(KAN) & Chloramphenicol(CM34) & Tetracycline(Tetra) & Ampicillin(Amp) & 60uL PYeaR(B) (1:50 dilution: Created 6:29:2016) & Minimal Media

Procedure:

1) Plate using LB Agar (See Autoclave Day: 6/2/2016):
   -(See Concentration Calculations in Data below)
   - 2 plates with Kanamycin
   - 12 plates with Chloramphenicol
   - 2 plates with Kanamycin/Tetracycline
   - 4 plates with Ampicillin
   - 2 plates with Kanamycin/ Chloramphenicol
2) Place all in 4degC freezer until further use
3) In 18 Large Glass Culture Tubes add:
   - 3 mL of Clean Media
   - 3uL of Chloramphenicol (1:1000 dilution)
   - 60uL of 2nd sub cultured PYeaR(B) (1:50 dilution: Created 6:29:2016)
4) In 1 Large Glass Tube add:
   - 3 mL of LB Broth
   - 3uL of Chloramphenicol (1:1000 dilution)
   - 60uL of 2nd subcultured PYeaR(B) (1:50 dilution: Created 6:29:2016)
5) Place in 37degC Shaker (Begin at 9:17am, End at 2:45 : @37degC @250rpm)
6) Keep in shaker until OD_{600} is measured between 0.4-0.6
7) Add predetermined nitrate concentrations (0mM, 0.4mM, 0.8mM, 1.2mM, 1.6mM, 2.0mM)
8) Place in 37degC Shaker (Aprox. 16 hrs @37degC @250rpm)
9) Place in 4degC refrigerator

Data: Below are the Concentration Calculations: (Step 1)

	Stock Concentration (mg/mL)	Dilution	Final Concentration (ug/mL)
Chloramphenicol	34	x1000	34
Kanamycin	50	x1000	50
Tetracycline	5	x400	12.5
Ampicillin	100	x1000	100

Results:
We created 22 plates to use in the future.

$\Delta SerA$ and B0015 Terminator Transformations

July 5th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: To Transform our Terminator and $\Delta SerA$

Materials:
Materials for Transforming

Procedure:
1) Transform $\Delta SerA$ cells (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)
2) Transforming B0015 Terminator with CM34 (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)
3) Plate each using streaking technique

Results: July 6th, 2016
The transformed cells did not grow and we believe it was do to a mistake in the streaking process. For Proper Streaking techniques See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016. We will perform PI again tomorrow.

$\Delta SerA$ into C.C. cells / Plating B0015 & NirS / Testing PYeaR with PBS Buffer _

July 6th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Turning $\Delta SerA$ into C.C. cells, Washing PYeaR Samples with PBS buffer and Plating B0015 & NirS

Materials:
Materials to Create C.C. cells (6/3/2016) & Plating & Autoclaved Water & DNA Samples

Procedure:
1) Turn $\Delta SerA$ & PYeaR DNA to C.C. cells (See E-Coli Chemical Bacteria Cells in Liquid Media / Competent Cells Preparation: 6/3/2016)
2) Aliquot 1 mL of each PYeaR DNA into separate eppendorf tubes.
3) In the transparent well plate (For overnight cell culture)
   -Add 30uL of suspended cells
   -Add 120uL of autoclaved water (Final Volume 150uL)
4) In Black well plate (For suspended cells)
   -Add 150 uL of suspended cells
5) Plate B0015 Terminator in LB Broth from 7/5/2016
6) Plate transformed $\Delta SerA$ cells from 7/5/2016
7) Place in 37degC incubator overnight

Results: July 7th, 2016
The colonies, when removed from incubator, were a bit on the small side, so we let them grow for a bit longer.

Inoculation of NirS & B0015 Term.

July 7th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Inoculating NirS and B0015 Terminator separately to get them ready to add together, which will happen on 7/8/2016

Materials:
Materials from Auto Clave Day (6/2/2016) & Inoculation

Procedure:
1) Inoculate NirS and B0015 Terminator (Created from their respective plates 7/6/2016)
2) Create new Agar and LB Broth (See Autoclave Day: 6/2/2016)

3A Assembly - Digestion and Ligation

July 8th, 2016
Project # 2
From Page # 15
To Page #
Originally an iGEM procedure
Recorded by: Brynne Schwabauer

Purpose: Prepping NirS Plasmid and B0015 Terminator to transform together

Note:
For linearized plasmid backbones provided by iGEM HQ, a plasmid backbone with an insert of BBa_J04450 was used as template. As a result any red colonies that appear during your ligation may be due to the template as a background. Digesting with Dpn1 before use should reduce this occurrence.

Materials:
Two Part Samples, A and B: Miniprepped DNA (in BioBrick RFC[10] plasmid backbones) & Linearized Plasmid Backbone (with a different resistance to the plasmid backbones containing your part samples) & 1uL of EcoRI-HF & 0.5uL of XbaI & 0.5uL of SpeI & 1uL of PstI & 7.5 uL of NEB Buffer 2.1 & 64.5uL of Autoclave Water

Procedure:
1) Add 2ul of digested Plasmid Backbone (25 ng) {For doubled amount 4uL}
2) Add equimolar amount of Part A {For doubled amount 2.54uL}
   - EcoRI-HF SpeI digested fragment (< 3 ul) Try for a 1:5 Plasmid:Insert
3) Add equimolar amount of Part B {For doubled amount 0.24uL}
   - XbaI PstI digested fragment (< 3 ul)
4) Add 1 ul T4 DNA Ligase Buffer (Do not use quick ligase)

5) Add 0.5 ul T4 DNA Ligase
6) Add until final volume of 10 uL {For doubled amount 1.72uL}
7) Ligate at room temperature for 1 hour
8) Transform with 1-2 ul of product (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)

To create each part: Digest all three reactions at 37degC for 30minutes then heat kill at 80degC for 20 minutes **(Under PCR iGEM Settings as Digestion 2)**

Enzyme Master Mix for Plasmid Backbone (25ul total, for 5 rxns)
- 2.5 ul NEB Buffer 2.1
- 0.5 ul EcoRI-HF
- 0.5 ul PstI
- 21.5 ul dH20

Enzyme Master Mix for Part A (25ul total, for 5 rxns)
- 2.5 ul NEB Buffer 2.1
- 0.5 ul EcoRI-HF
- 0.5 ul SpeI
- 21.5 ul dH20

Enzyme Master Mix for Part B (25ul total, for 5 rxns)
- 2.5 ul NEB Buffer 2.1
- 0.5 ul XbaI
- 0.5 ul PstI
- 21.5 ul dH20

Digest Plasmid Backbone
- Add 4 ul linearized plasmid backbone (25ng/ul for 100ng total)
- Add 4 ul of Enzyme Master Mix PB

Digest Part A
- Add 4 ul Part A (25ng/ul for 100ng total)
- Add 4 ul of Enzyme Master Mix A

Digest Part B
- Add 4 ul Part B (25ng/ul for 100ng total)
- Add 4 ul of Enzyme Master Mix B

Prepping NirS & B0015 Term. And Transforming them together

July 8th, 2016
Project # 2
From Page # 15
To Page #

Originally Recorded by: Brynne Schwabauer

Purpose: To transform together our NirS Plasmid and B0015 Terminator

Materials:

Materials from Glycerol Stock (6/2/2016) & Transformation (6/6/2016) &
3A HiFi Assembly Procedure (6/2/2016)

Procedure:

1) Create new Glycerol Stock (See Glycerol Stock: 6/29/2016)
2) Prep both NirS and B0015 Terminator samples (See Molecular Cloning / Plasmid DNA Preparation (Purple Kit): 6/8/2016)
3) 3A Assembly Procedure (See Digestion and Ligation: 7/8/2016)
4) Transform of NirS and B0015 Terminator together (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)
5) On Ampicillin resistant plates, plate:
   - Transformed NirS and B0015 Terminator
   - The Control (The plasmid without the inserts)
   - RBS (Plate 4, Well 16C)
6) To rehydrate RBS DNA from the iGEM kit: Plate 4, Well 16C
   -Puncture DNA well
   -Add 10uL of autoclave water
   -Mix by pipetting up and down gently

-Allow to sit for a minute

7) Incubate plates in 37degC overnight

Data: Data below was collected using the NanoDrop 2000 machine: (Step 2)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
NirS	1.84	2.19	250.9
B0015 Terminator	1.84	2.27	205.3
	Desired: >1.8	Desired: 1.5-1.9	

Data below are the Amount Calculations: (Step 3)
Insert A - NirS: 1.7kB
Insert B – B0015 Terminator: 129bp
Plasmid - pSB1A3: 2 kB

- Amount uL = [{Plasmid Mass * (X Insert Length kB / Plasmid Length kB) * (Plasmid: Insert ratio)} / (Concentration of X)]
  - [{ 25ng * (1.7 / 2) * (5)} / (83.6ng/uL)] = 1.27uL of A Insert
  - [{ 25ng * (0.13 / 2) * (5)} / (68.4ng/uL)] = 0.118uL of B Insert
  - 2uL of Plasmid

Results:
RBS Plate worked. The control and plated NirS / B0015 Term did not grow at all. The control samples shouldn't self-lygate; therefore the fact that no cells grew should be expected.

Testing Plasmid Backbone pSB1A3 and Prepping NirS & B0015 Term. again

July 11th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: To figure out if our plasmid backbone pSB1A3 was what prevented our cells from growing in our previous experiment (See Prepping NirS & B0015 Term. And transforming them together: 7/8/2016)

Materials:
Materials used in Electrophoresis (6/7/2016) & Inoculation & Ligation (7/8/2016) & - Transformation (6/6/2016) & Plating

Procedure:

1) Prepare 1% Electrophoresis Gel (See DNA purification by Electrophoresis: 6/7/2016)
2) Preform Electrophoresis on Plasmid Backbone samples:
   -Lane 1: DNA Ladder
   -Lane 2: Plasmid Backbone pSB1A3
3) Perform Ligation on B0015 Terminator and NirS (See 3A Assembly – Digestion and Ligation: 7/8/2016)
4) Transform together NirS and B0015 again (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
5) Plated NT on CM34 Plates
6) Place in 37degC incubator overnight

7) Create new plates using LB Agar (See Autoclave Day: 6/2/2016):
   - 8 plates with Chloramphenicol (1:1000)
   - 8 plates with Ampicillin (1:1000)
8) Place all plates in 4degC freezer until further use
9) Retransform samples from InterLab Study (See InterLab Measurement study: 6/29/2016 – 7/12/2016)
10) Inoculate and Plate Biobricks received today samples 1356005 and 135006 (On plates with CM34)
11) Placed in 37degC incubator

Results:

- ❖ We learned that the plasmid backbone is linearized which means that the control samples shouldn't be able to self-lygate; therefore the fact that no cells grew should be expected.
- ❖ The electrophoresis of the Plasmid Backbone came out to around 2.2kB, which is what it is supposed to be. That means that the Plasmid backbone is not malfunctioning yet.
- ❖ The NT cells grew!!

Inoculating NT / Prepping to add RBS DNA

July 12th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Inoculating NT to prepare for the addition of RBS DNA

Materials:
Materials used in Minimal Media (6/27/2016) & Inoculation

Procedure:

1) Inoculate NirS and Term. (NT)
2) Plate RBS on CM34 plate
3) Create Minimal Media (See M9 Glucose/ Leucine Minimal Medium: 6/27/2016)
4) Inoculate and Plate PyeaR on CM34 Plates

Transforming NT & RBS together

July 13th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Purifying and transforming together NT(NirS and B0015 Terminator) and the RBS DNA

Materials:
Materials used in the Molecular Cloning kit (Purple) (6/8/2016) & Inoculation & Digestion/Ligation (7/8/2016) & Transformation (6/6/2016) & Electrophoresis (6/7/2016) & Plating

Procedure:
1) Purify NT (NirS and B0015 Term.) using the Purple Kit (See Molecular Cloning / Plasmid DNA Preparation: 6/8/2016)
2) Perform Ligation on NT and RBS (See 3A Assembly – Digestion and Ligation: 7/8/2016)
3) Transform together NT and RBS (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
4) Plate on CM34 Plate and place in 37degC incubator overnight
5) Digest SerA sample (See Restriction Enzyme Digestion: 6/8/2016)

6) Purify SerA using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
7) Transform SerA (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)
8) Plate on CM34 Plate and place in 37degC incubator overnight
9) Perform PCR on NT plasmid (See Polymerase Chain Reaction: 6/7/2016)
   - As the NT Plasmid had such a high concentration the original plasmid was diluted with autoclave water in a 1:18 ratio, used 17 uL of autoclaved water)

Data: Data below was collected using the NanoDrop 2000 machine: (Step 1)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
NirS	1.75	1.32	500.6
RBS	1.82	2.01	98.8
	Desired: >1.8	Desired: 1.5-1.9	

Data below was collected using the NanoDrop 2000 machine: (Step 6 & 9)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
pSB1C3	--	--	7.0
SerA	--	--	4.6
NT Plasmid	1.81	1.66	500.2
	Desired: >1.8	Desired: 1.5-1.9	

Results:
The NT and RBS cells did not grow.

Transforming B0015 and SerA together

July 14th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: To bind together B0015 and SerA

Materials:
Materials used for Digestion/Ligation (7/8/2016) & Transformation (6/6/2016) & Plating & Inoculation

Procedure:
1) Perform Ligation on B0015 Terminator and SerA (See 3A Assembly – Digestion and Ligation: 7/8/2016)
2) Transform B0015 Terminator and SerA (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
3) Plate on CM34 Plate and place in 37degC incubator overnight

Inoculating New Primers

July 15th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Inoculating new primers

Materials:
Materials for Inoculation

Procedure:
1) Inoculate SB-prep-2Ea(R), SB-prep-3p-1 (F), NapDA(R), NapDA (F) and NapBC(R)-2 separately

Transforming B0015 Term. and SerA together again

July 18th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Purifying and transforming together B0015 Terminator and SerA

Materials:
Materials used in the Molecular Cloning kit (Purple) (6/8/2016) & Inoculation & Digestion/Ligation (7/8/2016) & Transformation (6/6/2016) & Electrophoresis (6/7/2016) & Plating

Procedure:

1) Purify B0015 Term. and SerA using the Purple Kit (See Molecular Cloning / Plasmid DNA Preparation: 6/8/2016)
2) Perform Ligation on B0015 Term. and SerA (See 3A Assembly – Digestion and Ligation: 7/8/2016)
3) Transform together B0015 Term. and SerA (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
4) Plate on CM34 Plate and place in 37degC incubator overnight
5) PCR NapDA (See PCR 1 Settings in Data)
6) PCR NapBC (See PCR 2 Settings in Data)
7) Purify NapDA and NapBC using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
   - Lane 1: DNA Ladder
   -Lane 3: Terminator Digest
   -Lane 5: SerA Digest
   -Lane 7 & 8: NapDA
   -Lane 10 & 11: NapBC
8) Transform pSB1C3 Backbone
9) Inoculate SerA

Data: Data below was collected using the NanoDrop 2000 machine: (Step 1)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
NT	1.83	2.09	188.4
NapBC	2.01	0.57	4.2
SerA	0.66	0.84	0.3
B0015 Term.	3.59	1.23	2.5
Redc SerA	3.08	0.82	2
	Desired: >1.8	Desired: 1.5-1.9	

Settings for PCR 1 (Step 5):

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	57	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	12 sec	29 times	3 min	inf.

Settings for PCR 2 (Step 6):

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	56	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	23 sec	29 times	3 min	inf.

Results: NapDA did not preform in the electrophoresis. We will try something new tomorrow

Purifying Nap DNA 12 & 345

July 19th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Purifying Nap DNA 12 & 345

Materials:
Materials for Inoculation & Digestion/Ligation (7/8/2016) & Transformation (6/6/2016) Electrophoresis (6/7/2016) & Plating

Procedure:

1) PCR Nap DNA 12 (See PCR 1 Settings in Data)
2) Purify Nap DNA 12 using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
3) Inoculate Nap DNA 12 and 345

Data: Data below is the settings used on the PCR: (Step 1)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	Gradient Temps.	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	49 sec	29 times	3 min	inf.

Step 1 Gradient Temperatures:
Sample 1 Nap 12: 65 degC
Sample 2 Nap 12: 62 degC
Sample 3 Nap 12: 59.1 degC
Sample 4 Nap 12: 52.9 degC
Sample 5 Nap 12: 50 degC

Data below was collected using the NanoDrop 2000 machine: (Step 3)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
Nap DNA 12	2.23	0.59	13.4
Nap DNA 345	1.85	0.35	19.2
pSerA	2	1.83	413.6
	Desired: >1.8	Desired: 1.5-1.9	

Purifying pSB1C3

July 20th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Purifying pSB1C3

Materials:
Materials for Inoculation & Electrophoresis (6/7/2016)

Procedure:

1) Purify pSB1C3 using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
2) Purify SerA and B0015 Term. using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
3) Inoculate NirS and B0015 Terminator

Data: Data below was collected using the NanoDrop 2000 machine: (Step 1)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
pSB1C3 Sample 1	1.85	1.48	129.8
pSB1C3 Sample 2	1.84	1.46	134
	Desired: >1.8	Desired: 1.5-1.9	

Data below was collected using the NanoDrop 2000 machine: (Step 2)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
pSB1C3	1.8	1.84	410.5
SerA	1.83	1.46	347.9
B0015 Term	1.84	2.02	221.4
	Desired: >1.8	Desired: 1.5-1.9	

HiFi DNA Assembly Protocol

July 21th, 2016
Project # 2
From Page # 15
To Page #
Originally an iGEM Procedure
Recorded by: Brynne Schwabauer

Purpose: Tying multiple strands of DNA together

Materials:
Autoclaved Water & NEBuilder HiFi DNA Assembly Master Mix & DNA fragments

Procedure:
1) Set up the following reactions on ice

	Recommended Amount of Fragments Used for Assembly		
	2–3 Fragment Assembly*	4–6 Fragment Assembly**	Positive Control †
Recommended DNA Ratio	vector:insert = 1:2	vector:insert = 1:1	
Total Amount of Fragments	0.03–0.2 pmols* X µl	0.2–0.5 pmols** X µl	10 µl
NEBuilder HiFi DNA Assembly Master Mix	10 µl	10 µl	10 µl
Deionized H_2O	10-X µl	10-X µl	0
Total Volume	20 µl † †	20 µl † †	20 µl

2) Incubate samples in a thermocycler at 50°C for 15 minutes (when 2 or 3 fragments are being assembled) or 60 minutes (when 4–6 fragments are being assembled). Following incubation, store samples on ice or at –20°C for subsequent transformation.
3) Transform NEB 5-alpha or 10-beta Competent *E. coli* cells (provided in the cloning kit, bundle or purchased separately from NEB) with 2 µ l of the assembled product, following the transformation protocol.

Notes:
- Optimized cloning efficiency is 50–100 ng of vector with 2-fold excess of inserts. Use 5 times more insert if size is less than 200 bp. Total volume of unpurified PCR fragments in the assembly reaction should not exceed 20%.
- *Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).*

Tying together NapDABC & pSB1C3 and B0015 & pSerA

July 21st, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Tying together NapDABC and pSB1C3 and B0015 & pSerA

Materials:

Materials for Inoculation & Digestion/Ligation (7/8/2016) & Transformation (6/6/2016) & Electrophoresis (6/7/2016) & Restriction Enzyme Digestion (6/8/2016) & Plating & Molecular Cloning kit (Purple) (6/8/2016)

Procedure:

1) Digest B0015 Term. and pSerA (Restriction Enzyme Digestion: 6/8/2016)
2) Purify B0015 Term and pSerA using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
3) Perform Ligation on B0015 Term. and pSerA (See 3A Assembly – Digestion and Ligation: 7/8/2016)
4) Transform together B0015 Term. and pSerA (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
5) Plate on CM34 Plate and place in 37degC incubator overnight
6) PCR and purify NapA using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
7) Perform 3A HiFi Assembly on NapDABC and pSB1C3 **on ICE** (See HiFi DNA Assembly: 7/21/16)
   - 10uL HiFi Master Mix
   -0.19uL pSB1C3 Sample 2
   -0.53uL NapDA
   -2.01uL NapA
   -6.52uL NapBC
   -0.75uL H20
   - ❖ Incubate for 15 min at 55degC
   - ❖ Store at -20degC until further use
8) Transform together NapDABC and pSB1C3 (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
9) Plate on CM34 Plate and place in 37degC incubator overnight
10) Perform Miniprep on inoculated sample of NirS and Term. (Cannot do any more with the safety go-ahead)

Results: NirS didn't inoculate properly…maybe it's a sign we shouldn't be using it…. Or a spy amongst the ranks…

Data: Data below was collected using the NanoDrop 2000 machine: (Step 1)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
SerA	1.89	0.34	31.8
	Desired: >1.8	Desired: 1.5-1.9	

Data below was collected using the NanoDrop 2000 machine: (Step 2)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
SerA	1.96	0.04	9.8

B0015 Term	1.28	0.65	19.2
	Desired: >1.8	Desired: 1.5-1.9	

Data below was collected using the NanoDrop 2000 machine: (Step 6)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
.4	1.78	1.73	268.4
	Desired: >1.8	Desired: 1.5-1.9	

Results: We now have a way to quantify nitrate levels!!!!

PCR NapDA, NapA & NapBC and Inoculate SerA

July 22nd, 2016
Project # 2
From Page # 15

To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Prepping NapDA, NapA and NapBC

Materials:
Materials for Inoculation & Electrophoresis (6/7/2016) & PCR (6/6/2016)

Procedure:
1) PCR NapA, NapBC, NapDA (See PCR 1 Settings in Data)
2) Purify NapA, NapBC, NapDA using Electrophoresis (See DNA Purification by Electrophoresis, Steps 1-12: 6/7/2016)
3) Inoculate SerA and B0015 Term.
4) Place in 37deg Incubator overnight
5) Leave in 4degC fridge over the weekend

Data: Data below was collected using the NanoDrop 2000 machine: (Step 2)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
NapA	2.75	0.23	12.8
NapBC	1.89	0.89	52.4
NapDA	1.89	1.18	66.8
	Desired: >1.8	Desired: 1.5-1.9	

Prepping pSerA & B0015 Term. and pSerA & RBS

July 25th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Prepping pSerA & B0015 and pSerA & RBS

Materials:
Materials used for Restriction Enzyme Digestion (6/8/2016) & CaCl DNA Recovery Kit (Yellow kit) (6/8/2016) & Glycerol Stock (6/29/2016) & HiFi DNA Assembly (6/29/2016) & Transformation (7/8/2016) & 3 uL Cutsmart & 3 uL EcoRI-HF & 1 uL Xba1 & 2 uL Spe1 & 17.38 uL of H2O & 2.5 uL of SerA & B0015 Term & 3 uL of PYeaR & 2 uL of RBS & 5uL NapA & 10uL HiFi Master Mix & 0.26uL pSB1C3 Sample 2 & 0.22uL NapDA & 0.73uL NapBC

Procedure:
1) On Sunday (7/24/2016) inoculate pSerA and RBS
2) Place in 37degC incubator overnight
3) Perform column Purification of pSerA and B0015 Term (using the Yellow kit)
4) Digest pSerA & B0015 Term together, PYeaR and RBS separately
5) Place all mixtures in a 37degC water bath for 3 hours.
6) Remove mixtures and place in refrigerator at 4degC overnight
7) Create Glycerol Stock using pSerA & B0015 Term. (See Glycerol Stock: 6/29/2016)
8) Perform HiFi Assembly on NapDABC and pSB1C3 **on ICE** (See HiFi DNA Assembly: 7/21/16)
   - 10uL HiFi Master Mix
   -0.26uL pSB1C3 Sample 2
   -0.22uL NapDA
   -5uL NapA
   -0.73uL NapBC
   -3.78uL H20
   - ❖ Incubate for 15 min at 55degC
   - ❖ Store at -20degC until further use
9) Transform together NapDABC and pSB1C3 (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
10) Plate on CM34 Plates and place in 37degC incubator overnight

To create each part of the Digest:

SerA & B0015 (3.5kb)
- 1 uL Cutsmart
- 1 uL EcoRI-HF
- 1 uL Xba1
- 4.5 uL of H2O
- 2.5 uL of SerA & B0015 Term

PYeaR (146bp)
- 1 uL Cutsmart
- 1 uL EcoRI-HF
- 1 uL Spe1
- 4 uL of H2O
- 3 uL of PYeaR

RBS (98bp)
- 1 uL Cutsmart
- 1 uL EcoRI-HF
- 1 uL Spe1
- 5 uL of H2O
- 2 uL of RBS

Data: Data below was collected using the NanoDrop 2000 machine: (Step 3)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)

pSerA & B0015 Term.	1.84	2.19	449.1
	Desired: >1.8	Desired: 1.5-1.9	

Data below was collected using the NanoDrop 2000 machine: (Step)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
pSerA & B0015 Term.	1.88	0.23	325.2
RBS	1.78	0.81	30.4
PYeaR	2.07	0.16	13.6
	Desired: >1.8	Desired: 1.5-1.9	

Results: We have a Nitrate sensitive promoter and are looking to finish our nitrate reducatase today. HiFi Assembly failed again

NEB HiFi Assembly

July 26th, 2016
Project # 2
From Page # 15
To Page #
Originally a New England BioLabs Procedure
Recorded by: Brynne Schwabauer

Purpose: To try out a new type of HiFi Assembly procedure as our 3A hasn't worked so far.

Materials:
50uL of C.C. cells

Procedure:

1) Thaw chemically competent cells on ice.
2) Transfer 50 µl of competent cells to a 1.5 ml microcentrifuge tube (if necessary).
   -If the chemically competent cells are from New England Biolabs, add 2 µl of assembled product to NEB competent cells and go to step 4 directly.
   -If competent cells are purchased from other manufacture, dilute assembled products 4-fold with H_2O prior transformation. This can be achieved by mixing 5 µl of assembled products with 15 µl of H_2O. Add 2 µl of the diluted assembled product to competent cells.
3) Mix gently by pipetting up and down or flicking the tube 4–5 times. Do not vortex.
4) Place the mixture on ice for 30 minutes. Do not mix.
5) Heat shock at 42°C for 30 seconds.* Do not mix.
6) Transfer tubes on ice for 2 minutes.
7) Add 950 µl of room temperature SOC media* to tubes.
8) Place the tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
9) Warm selection plates to 37°C.
10) Spread 100 µl of the cells onto the plates with appropriate antibiotics.
    - Use Amp plates for positive control sample.
11) Incubate plates overnight at 37°C.

New C.C. cells and NEB HiFi Assembly

July 26th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Try to a new HiFi Assembly procedure with different C.C. cells

Materials:
Materials used for Plating & NEB HiFi DNA Assembly (7/26/2016) & Transformation (7/8/2016) & Electrophoresis (6/7/2016) & PCR (6/6/2016)

Procedure:
1) PCR NapA
2) Preform Electrophoresis on NapA Primer:
3) Use 1% buffer (See DNA Purification by Electrophoresis: 6/7/2016)
   -Did not continue with NapA as Nanodrop results were horrific. (See in Data Below)
4) Perform NEB HiFi Assembly on NapDABC and pSB1C3 **on ICE** (See NEB HiFi DNA Assembly: 7/26/16)
5) Transform together NapDABC and pSB1C3 (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
6) Plate on CM34 Plates and place in 37degC incubator overnight
7) Ligation of PYeaR and SerA & Term. Dilute SerA & Term. to 27 ng/uL (1/12th dilution: 1uL of DNA / 11uL H2O)
   - Mix together
     -1 uL of diluted SerA & Term.
     -0.21 uL of PyeaR
     -0.5 uL of 10X T4 Ligase Buffer
     -0.25 uL of T4 Ligase
     -3.04 uL of H2O
   - Leave mixture at room temp. for 1 hour
8) Plate on CM 34 plates and place in 37degC overnight

Data: Data below was collected using the NanoDrop 2000 machine: (Step 3)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
NapA	7.77	.01	13.2
	Desired: >1.8	Desired: 1.5-1.9	

Results: The NEB HiFi Assembly worked!!!!

Inoculate PYeaR and SerA

July 27th, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Inoculate PYeaR and SerA

Materials:
Materials used for Inoculation

Procedure:
1) Inoculate PYeaR and SerA.
2) Place in 37degC shaker overnight

Plating and Inoculating PyeaR-GFP & PyeaR-SerA & B0015 Term & NapDABC

August 1st, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Plating and Inoculating PyeaR GFP

Materials: Materials used for Inoculation & Plating & Transforming (7/8/2016)

Procedure:

1) Plate B0015 Terminator & NapDABC
2) Inoculate NapDABC & PyeaR-SerA & PyeaR-GFP
3) Transform and plated pSB3C5 (Plate 4, Well 4D) & pSB4C5 (Plate 4, Well 4F)
4) Place in 37degC incubator overnight

Isolate and Purify NapDABC & PyeaR-SerA

August 2nd, 2016
Project # 2
From Page # 15
To Page #
Originally Recorded by: Brynne Schwabauer

Purpose: Isolating and Plating NapDABC & PyeaR-SerA

Materials:

Materials used for Inoculation & Plating & Molecular Cloning kit (Purple) (6/8/2016) & Transforming (7/8/2016) & 2uL of NapDABC & 1uL of Pst1-HF & 1uL of Cutsmart & 23.25 uL of H2O & 1uL of Eco-R1-HF & 2uL of VF2 & 2uL of VR & 0.75uL of Primer Mix & 1uL of PyeaR-SerA DNA (10ng) & 2.5uL of 10X KOD Buffer & 1.5uL of MgSO4 & 0.5uL of KOD Po1 & 2.5uL of dNTPs

Procedure:

1) Isolate and Purify NapDABC (See Molecular Cloning / Plasmid DNA Preparation: 6/8/2016)
2) Digest NapDABC (Restriction Enzyme Digestion: 6/8/2016)
   - ❖ Mix Together(10uL Final Volume):
     -2uL of NapDABC
     -1uL of Pst1-HF
     -1uL of Cutsmart
     -1uL of Eco-R1-HF
     -5uL of H2O
   - ❖ Heat at 37degC for 1 hour
3) Inoculate NapDABC & B0015 Term.
4) PCR PyeaR-SerA (1.7kB)
   - ❖ Dilute PyeaR-SerA to 10ng/uL
   - ❖ Make Diluted Primer Mix:
     - 2uL of VF2
     - 2uL of VR
     - 2uL of H2O
   - ❖ Mix Together:
     - 0.75uL of Primer Mix
     - 1uL of PyeaR-SerA DNA (10ng)
     - 2.5uL of 10X KOD Buffer
     - 1.5uL of MgSO4
     - 0.5uL of KOD Po1
     - 2.5uL of dNTPs
     -16.25uL of H2O
   - ❖ PCR (See Settingsfor PCR in Data below)
5) Preform Electrophoresis on NapA Primer:
6) Use 1% buffer (See DNA Purification by Electrophoresis: 6/7/2016)
7) Sequence PyeaR-SerA (VF2: AFG119 & Reverse: AFG 118)
8) Transform ΔSerA & Pyear-SerA together (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
   - For PyeaR-SerA add 0.34uL to 40uL of ΔSerA C.C. Cells
   - For RBS DNA added approx. 2uL (There was not enough to have the full 3uL) to 40uL of Genehog C.C. Cells
9) Inoculate PyeaR-SerA & pSB3C5 & pSB4C5

Data: Data below was collected using the NanoDrop 2000 machine: (Step 1)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
SerA & PyeaR Sample 1	1.73	0.76	92.0

SerA & PyeaR Sample 2	1.88	0.40	143.1
	Desired: >1.8	Desired: 1.5-1.9	

Settings for PCR (Step 5):

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	57	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	34 sec	29 times	3 min	inf.

Data below was collected using the NanoDrop 2000 machine: (Step 6)

Test Tube	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
SerA & PyeaR	1.78	1.44	293.8
NapDABC	1.79	1.39	464.1
	Desired: >1.8	Desired: 1.5-1.9	

Isolate/ Digest/ 3A HiFi/ Griess Prep

August 3rd, 2016
Project # 2
From Page # 15
To Page #
Recorded by: Joshua Mueller

Purpose: Isolating and Purifying Backbones and NirS

Materials: Materials used for Inoculation & Molecular Cloning kit (Purple) (6/8/2016) & 3A Assembly (7/21/16) & Restriction Enzyme Digestion (6/8/2016) & Transformation (7/8/2016) & Ligation

Procedure:

1) Isolate and Purify RBS & NapDABC & B0015 Terminator & PyeaR-SerA & pSB3C5 & pSB4C5 (See Molecular Cloning / Plasmid DNA Preparation: 6/8/2016)
2) Perform Digestion and Ligation (See 3A Assembly – Digestion and Ligation: 7/8/2016)
   - ❖ Dilute NapDABC to 25 ng/uL - 1uL DNA: 22uL H2O
   - ❖ Dilute B0015 Term. to 25 ng/uL - 1uL DNA: 11uL H2O
   - ❖ For Digest mix together: NapDABC: 2.15kb / B0015 Term: 2.15kb / pSB1A3: 2.15kb
     - 2 uL of Plasmid
     - 7.8uL of Dilute NapDABC
     - 0.29uL of Dilute B0015 Terminator
     - 1.5uL of T4 Buffer
     - 0.5uL of DNA Ligase
     -2.9uL of H2O
3) Perform 3A Assembly on NapDABC B0015 Terminator & PyeaR-SerA & pSB1A3 (See 3A DNA Assembly: 7/21/16)
4) Digest PyeaR-SerA & pSB3C5 & pSB4C5 (Restriction Enzyme Digestion: 6/8/2016)
5) Inoculate NirS-Terminator & RBS & pSB4C5 & PyeaR-serA in LB Broth
6) Transform and plate NapDABC-term (Used DNA from 8/3/16) (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
7) Prepare Griess reagent and use plate reader

Data: Data below was collected using the NanoDrop 2000 machine: (Step 1)

	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
PyeaR-SerA	1.84	2.2	502.8
pSB4C5	1.84	2.2	425
pSB3C5	1.84	2.19	580.4
NapDABC	1.84	2.18	510.5
B0015 Term	1.83	2.22	315.2
RBS	1.85	2.13	104.9
	Desired: >1.8	Desired: 1.5-1.9	

Data: Data below was collected using the NanoDrop 2000 machine: (Step 3)

	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
PyeaR-SerA	1.83	1.8	51.8

pSB4C5	2.66	0.1	4
pSB3C5	2.26	0.34	3.8
Overnight part pSB3C5	1.83	1.83	1175.9
	Desired: >1.8	Desired: 1.5-1.9	

This concentration for overnight pSB3C5 was ridiculous because 2/3 of the water in the eppendorf tube had evaporated overnight. 6uL of pSB3C5 left

C.C. cell & M9 prep & NapDABC seq & Digestion

August 4th, 2016
Project # 2
From Page # 66
To Page #
Recorded by: Joshua Mueller

Purpose: To prepare NapDABC for continued use

Notes: For Sequencing: AFG113 used VF2 primer, AFG114 used VR primer

Materials: Materials used for Inoculation & Molecular Cloning kit (Purple) (6/8/2016) & Assembly (7/21/16) & Restriction Enzyme Digestion (6/8/2016) & Transformation (7/8/2016) & Digestion/Ligation (7/8/2016) & Electrophoresis (6/7/2016) 3A

Procedure:

1) PCR NapDABC DNA (from 8/2/16) (See PCR Settings in Data)
2) Purify NapDABC (See Molecular Cloning / Plasmid DNA Preparation: 6/8/2016)
3) Send NapDABC primers for Sequencing (See Molecular Cloning / DNA Sequencing: 6/13/16)
4) Digest PyeaR-serA & pSB3C5, and pSB4C5 DNA from 8/3/16 using EcoR1 & Pst1 (See Restriction Enzyme Digestion: 6/8/2016)
5) Gel purify digestion products (See DNA Purification by Electrophoresis: 6/7/2016)
   - PyeaR : 1.5kb - pSB4C5: 3.2kb -pSB3C5: 2.7kb
6) Ligate purified products for 1 hour (See 3A Assembly – Digestion and Ligation: 7/8/2016)
7) Prepare Genehogs C.C. cells (See E-Coli Chemical Bacteria Cells in Liquid Media / Competent Cells Preparation: 6/7/16)
8) Prepare M9 media (See M9 Glucose/Leucine Minimal Medium: 6/27/16)

Data: Data below is the settings used on the PCR: (Step 1)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
DegC	94	94	57	70	Repeat Steps 2-4:	70	4
Time	2 min	30 sec	15 sec	12 sec	29 times	3 min	inf.

Data below was collected using the NanoDrop 2000 machine: (Step 1)

	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
NapDABC	1.87	0.16	117.8
	Desired: >1.8	Desired: 1.5-1.9	

Digestion and Purification of PyeaR-SerA

August 8th, 2016
Project # 2
From Page # 66
To Page #
Recorded by: Brynne Schwabauer

Purpose: Digestion and Purification of PyeaR-SerA

Notes: For Sequencing: AFG124: NapDABC Primer 2, AFG125: NapDABC Primer 3 and AFG126: NapDABC Primer 4

Materials: Materials for Inoculation & Restriction Enzyme Digestion (6/8/2016) & Transformation (7/8/2016) & Digestion/Ligation (7/8/2016) & Electrophoresis (6/7/2016) & 3A Assembly (7/21/16)

Procedure:

1) Digest PyeaR-serA & pSB3C5, and pSB4C5 DNA from 8/3/16 using EcoR1 & Pst1 (See Restriction Enzyme Digestion: 6/8/2016)
2) Gel purify digestion products (See DNA Purification by Electrophoresis: 6/7/2016)
3) Ligate products for 1 hour (See 3A Assembly – Digestion and Ligation: 7/8/2016)
4) Send NapDABC primers for Sequencing (See Molecular Cloning / DNA Sequencing: 6/13/16)

Data: Data below was collected using the NanoDrop 2000 machine: (Step 2)

	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
PyeaR-SerA	1.85	0.13	59.0
pSB4C5	1.84	0.14	90.3
pSB3C5	1.78	0.19	133.6
	Desired: >1.8	Desired: 1.5-1.9	

Transformation of NapDABC + B0015 Term.

August 9th, 2016
Project # 2
From Page # 66
To Page #
Recorded by: Brynne Schwabauer

Purpose: Transformation of NapDABC + B0015 Term.

Materials: Materials for Plating & Inoculation & Transformation (6/6/2016) & Transformation (7/8/2016)

Procedure:

1) Transform together NapDABC + B0015 Term (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)
2) Transform separately PyeaR- SerA-3C5 & PyeaR- SerA-4C5 (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)
3) Inoculate NapDABC + B0015 Term & Genehogs Cells
4) Plate $\Delta SerA$

Digest and Ligate NapDABC + B0015 Term. + pSB1A3

August 10th, 2016
Project # 2
From Page # 66
To Page #
Recorded by: Brynne Schwabauer

Purpose: Digest and Ligate NapDABC + B0015 Term. + pSB1A3 together

Materials: Materials for Inoculation & Transformation (6/6/2016) & 3A Assembly (7/8/2016)

Procedure:

1) Complete creation of C.C. cells (See Transformation of Chemical Competent Cells with Plasmid DNA: 6/6/2016)
2) Digest and Ligate NapDABC (50ng/uL) + B0015 Term (25ng/uL) + pSB1A3 (25ng/uL) (See 3A Assembly – Digestion and Ligation: 7/8/2016)
3) Inoculate PyeaR- SerA-3C5 & PyeaR- SerA-4C5 & $\Delta SerA$ (Control) in Minimal Media

Inoculation of NAP-T

August 11th, 2016
Project # 2
From Page # 66
To Page #
Recorded by: Brynne Schwabauer

Purpose: Inoculation of NAP-T and discussion on how to characterize our genes

Materials: Materials for Inoculation

Procedure:

1) Measure RBS DNA Concentration (10uL) (See NanoDrop 2000 in Data below)
2) Inoculate NapDABC and B0015 Term. in Minimal Media
3) Discuss PyeaR-serA / nap/ RBS testing

Data: Data below was collected using the NanoDrop 2000 machine: (Step 1)

	Nucleic Acid/ Protein A260/A280	Nucleic Acid/ Salt A260/A230	Concentration (ng/uL)
RBS	1.79	1.41	261.1
	Desired: >1.8	Desired: 1.5-1.9	

Tying together Nap-T & RBS & pSB1C3

August 12th, 2016
Project # 2
From Page # 66
To Page #
Recorded by: Brynne Schwabauer

Purpose: Tying together NapDABC-Terminator & RBS & pSB1C3

Materials: Materials for Inoculation & Transformation (7/8/2016) & 3A Assembly (7/8/2016) & Molecular Cloning kit (Purple) (6/8/2016)

Procedure:

1) Purify NapDABC-Term & pSB1A3 & RBS (See Molecular Cloning / Plasmid DNA Preparation: 6/8/2016)
2) Perform 3A HiFi Assembly on NapDABC-Term (60ng/uL) + RBS (25ng/uL) + pSB1A3 (25ng/uL) **on ICE** (See HiFi DNA Assembly: 7/21/16)
3) Transform together NapDABC-Term & pSB1A3 & RBS (See Prepping NirS & B0015 Term. And Transforming them together: 7/8/2016)

DNA Used:

PET30b –mCherry
PYeaR-GFP (Plate 1, Well 4B)
B0015 Terminator (Plate 3, Well 3F)
NirS
Jw2880 ∆ SerA
RBS (Plate 4, Well 16C)
1356005
1356006

Our DNA Created:

NT – NirS and B0015 Terminator
NapDABC – NapA, NapD, NapBC

NapA-R
NapA-F
NapBC-R
NapBC-F
NapAD

Plasmid Backbones:
pSB1A3
pSB1C3

Primers:
SLIC – NdeI-mCherry-F
SLIC – SacI-mCherry-R
iGEM-VF-2
iGEM-VR

Antibiotics:
Chloramphenicol (CM34)
Kanamycin (KAN)
Tetracycline (Tetra)
Ampicillin (AMP)

PyeaR Promoter

***E. coli* Strains:**
Genehog
MG1655
NEB 5-Alpha Comp E. coli (HE)

InterLab Measurement Study

June 29th, 2016 – July 13th, 2016

Project # X
From Page #
To Page #
Originally an iGEM procedure
Recorded by: Brynne Schwabauer

Purpose: To measure GFP fluorescence that will result in common, comparable units for teams to test out. How close can the numbers be when fluorescence is measured all around the world?

Materials:
-Materials needed for Transformation - Materials needed for DNA Competency Test

Procedure:
1) Thaw out Genehog competent cells on ice: This may take 10-15min for a 260µl stock.
2) To a 2ml tube pipette 50µl of competent cells Keep all tubes on ice.
3) Pipette 1µl of resuspended DNA into 2ml tube: Gently pipette up and down to mix
4) Pipette 1µl of control DNA into 2ml tube: Pipette 1µl of 10pg/µl control into your control transformation.
5) Incubate tubes on ice for 30min: Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.
6) Heat shock tubes at 42°C for 1 min: Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.
7) Incubate on ice for 5min

8) Add 200µl SOC media to each transformation: SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.
9) Incubate tubes at 37°C shaker or 2 hours
10) Pipette each transformation on two petri plates for a 20µl and 200µl plating
11) Incubate transformations overnight (14-18hr) at 37°C
12) Pick single colonies from transformations: do a colony PCR to verify part size, make glycerol stocks, grow up cell cultures and miniprep.
13) Count colonies for control transformation: Count colonies on the 20µl control plate and calculate your competent cell efficiency. Competent cells should have an efficiency of 1.5x10^8 to 6x10^8 cfu/µg DNA. (See DNA Competency Test: 6/14/2016)

Results:

June 29th, 2016
Transformed and plated cells: but colonies did not grow on either 20 or 200 uL plates. Must transform again
June 30th, 2016
Obtained LUMOX standard curve according to iGEM protocol
July 6th, 2016
Obtained FITC standard curve according to iGEM protocol
July 11th, 2016
Retransforming and plating cells again today. The cells plated previously ate through the antibiotic thus allowing other bacteria to grow. All samples became mold.

JW2880 -